# Synthesis of Conformationally Restricted β-Turn Mimics

Een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

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door

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geboren op 23 september 1976 te Bovenkarspel

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*Le paradis terrestre est où je suis*
*(Paradise is where I am)*

*Voltaire*

# *Contents*

# *List of Abbreviations*

| | |
|---|---|
| AA | amino acid |
| Abu | aminobutyric acid |
| Ac | acetyl |
| Acm | acetamidomethyl |
| Alg | allylglycine |
| app | appearant |
| aq | aqueous |
| Ar | aromate |
| BINAP | 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl |
| Bn | benzyl |
| Boc | *tert*-butoxycarbonyl |
| bs | broad singlet (NMR) |
| Bz | benzoyl |
| Cbz | carbonyl benzyloxy |
| CI-MS | chemical ionisation mass spectroscopy |
| COSY | correlation spectroscopy |
| Cy | cyclohexane |
| d | doublet (NMR) |
| DAP | 2,3-diaminopropanoic acid |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| DCC | *N*,*N*-dicyclohexylcarbodiimide |
| de | diastereomeric excess |
| Dha | dehydroalanine |
| Dhb | dehydrobutyrine |
| DIC | *N*,*N*-diisopropylcarbodiimide |
| DiPEA | *N*,*N*-diisopropylethylamine |
| DMAP | 4-(dimethylamino)pyridine |
| DMB | dimethoxybenzyl |
| DMF | *N*,*N*-dimethylformamide |
| DMS | dimethyl sulfide |
| DMSO | dimethyl sulfoxide |
| e.g. | exempli gratioa (for the sake of example) |
| ee | enantiomeric excess |
| EI-MS | electron impact mass spectroscopy |
| Et | ethyl |
| *et al.* | et aliae (and others) |
| EtOAC | ethylacetate |
| FAB-MS | fast atom bombardment mass spectroscopy |
| Fmoc | 9-fluorenylmethyloxycarbonyl |
| GMO | genetically modified organism |
| Grb2 | growth factor receptor-bound protein 2 |
| HBS | hydrogen-bond surrogate |
| hhMpg | bishomomethylpropargylglycine |
| hhPrg | bishomopropargylglycine |
| hMpg | homomethylpropargylglycine |
| HOBt | *N*-hydroxybenzotriazole |
| HPLC | high pressure liquid chromatography |
| Hse | homoserine |
| *i.e.* | *id est* (that is) |
| $IC_{50}$ | concentration *in vivo* with 50% inhibition effect |
| IR | infrared |
| LCMS | liquid chromatography mass spectroscopy |
| LDA | lithium diisopropylamide |
| M | molar |
| MALDI-TOF | matrix-assisted laser desorption ionization-time of flight mass spectroscopy |
| MC4 | melanocortin 4 receptor |
| Me | methyl |
| Mes | mesityl |
| MOM | methoxymethyl |
| Mpg | methylpropargylglycine |
| Ms | methanesulfonyl |
| MS | molecular sieves |
| NBS | *N*-bromosuccinimide |
| NMR | nuclear magnetic resonance |
| NOESY | nuclear Overhauser effect spectroscopy |
| OSu | hydroxysuccinimide |
| PG | protecting group |
| Ph | phenyl |
| ppm | parts per million |
| Prg | propargylglycine |
| pTSA | *p*-toluenesulfonic acid |
| PyBOP | (benzotriazol-1-yloxy) tripyrrolidinephosphonium hexafluorophosphate |
| q | quartet (NMR) |
| Ra-Ni | Raney nickel |
| RCAM | ring-closing alkyne metathesis |
| RCM | ring-closing metathesis |

| | |
|---|---|
| Rf | retention factor |
| RNAse | ribonuclease |
| ROESY | rotating frame Overhauser effect spectroscopy |
| RP | reverse phase |
| RT | room temperature |
| s | singlet (NMR) |
| t | triplet (NMR) |
| TBAF | tetrabutyl ammonium fluoride |
| $^{t}$Bu | *tert*-butyl |
| Tf | trifluoromethanesulfonyl |
| TFA | trifluoroacetic acid |
| TFFH | tetramethylfluoroformamidinium hexafluorophosphate |
| THF | tetrahydrofuran |
| THP | tetrahydro-2H-pyran |
| THP | tetrahydropyran |
| TLC | thin layer chromatography |
| TMS | trimethylsilyl |
| TMSE | trimethylsilylethyl |
| Trt | trityl |
| Ts | *p*-Toluenesulfonyl |
| UV | ultraviolet |
| *viz.* | *videlicet* (it is permitted to see) |

| | |
|---|---|
| **Paranimfen**: | Bas W. T. Gruijters |
| | Christien A. Schortinghuis |

# 1 PROTEINS AND CONFORMATIONAL RESTRICTION

## 1.1 Introduction

Nature's complexity culminated in the evolution of 'living' organisms. The basis of these organisms is formed by complex catalysts which are necessary to drive metabolics along complicated pathways. Despite the variety of function of these catalysts they consist of repetitive chains of a limited set of 20 amino acid building blocks. A larger set is provided by Nature through post-translation modifications.

Chains of amino acids fold into specific three-dimensional shapes. This folding eventually determines to a large extent the biological activity of the sequence. Two of the basic secondary structural elements that occur locally in protein structures are α-helices and ß-sheets. While α-helical peptides have been studied in great detail and considerable advances have been made in understanding the energetics of helix formation,[1] ß-sheets have until recently received less attention. The ß-sheet classifications can be further characterized as shown in Table 1 for turns that occur in ß-sheet structures (parallel linear sequences do not necessarily end in a turn).

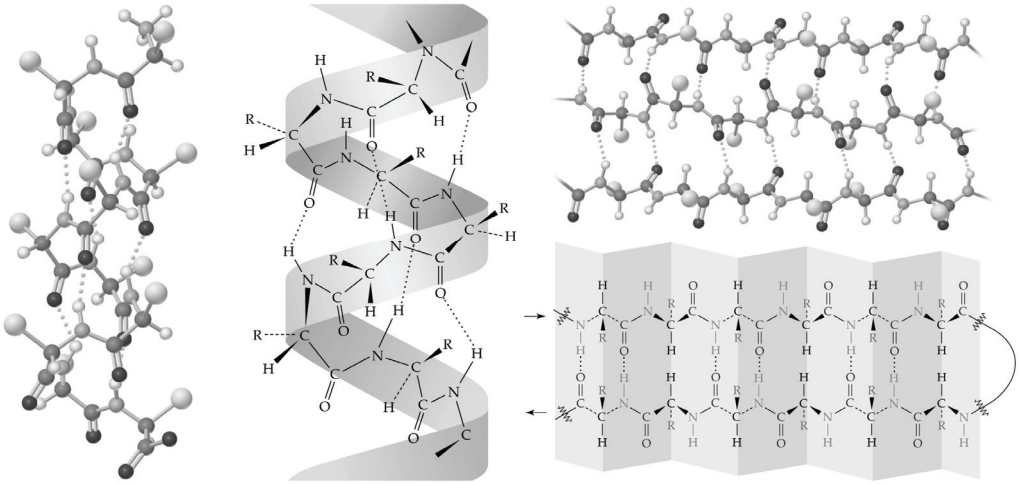
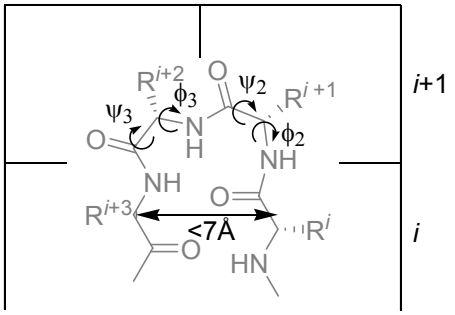


**Figure 1**. *α-Helix and β-turn*

**Tabel 1**

| Turn type | φ2 | ψ2 | φ3 | ψ3 | |
|---|---|---|---|---|---|
| I | −60° | −30° | −90° | 0° | *i*+2 |
| I` | 60° | −30° | 90° | 0° | |
| II | −60° | 120° | 80° | 0° | |
| II` | 60° | 120° | −80° | 0° | *i*+3 |
| III | −60° | −30° | −60° | −30° | |
| III` | 60° | −30° | 60° | −30° | |
| IV | Other | | | | |




The α-helices and ß-sheets/turns are interesting because of the relation between their structure and biological activity. These secondary structures are encountered in proteins, where they are involved in recognition and binding. Mimics of these secondary motifs can be useful in determining structure/activity relations, but naturally it is important for a peptide to retain its conformational features. Conformational restriction is a way to provide insight in structural-activity relationships.

Conformational restrictions includes stabilizing peptide motifs. For example, incorporation of covalent or non-covalent linkages between amino acid side chains could give enhanced stability. Examples include salt bridges,[2] lactams,[3] disulfide bridges,[4] hydrophobic interactions,[5] metal ligation between natural[6] and unnatural[7] amino acids and lipid attachments.[8]

In recent years, there has been an exploding interest in the use of metathesis, culminating in the Nobel Prize of Chemistry 2005 for Chauvin, Grubbs and Schrock.

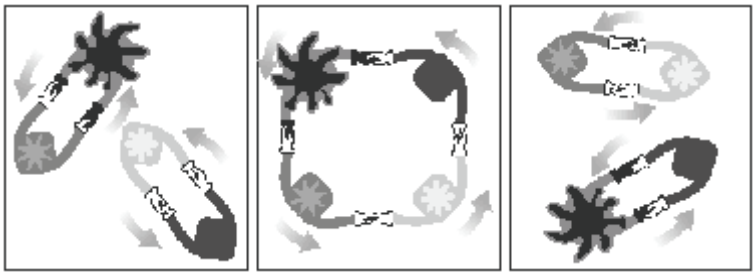


**Figure 2**. *Schematic representation of metathesis: exchanging dance partners (The Chauvin[9] mechanism.)*

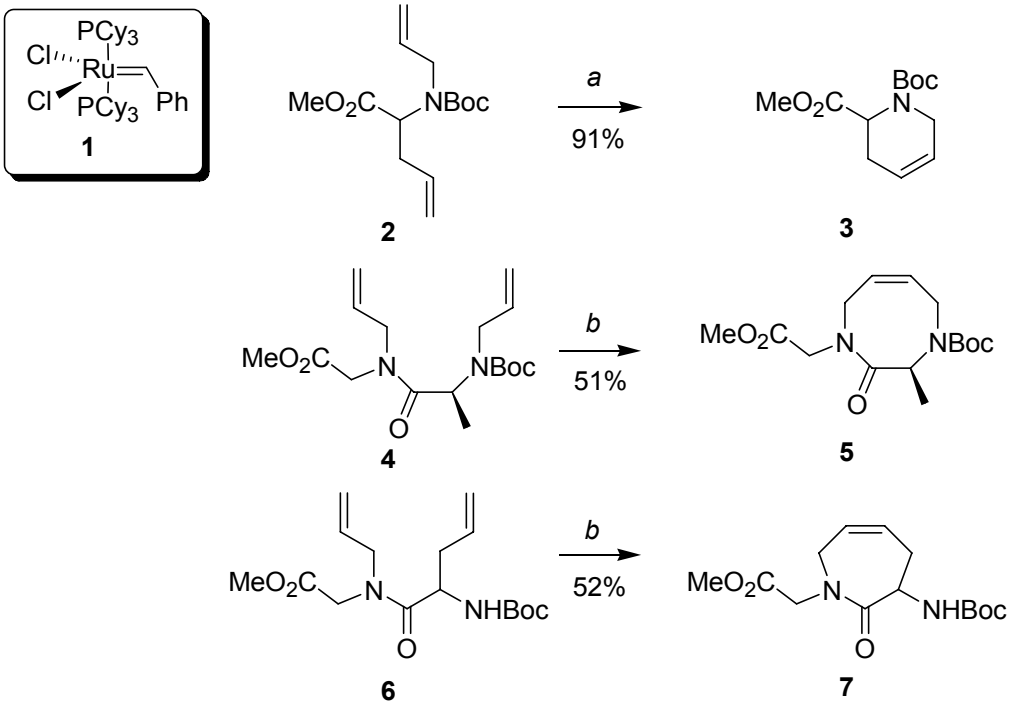
One of the features of metathesis is that it is capable of forming artificial macrocylic rings and carbon bridges (ring-closing metathesis or RCM), which can be applied to introduce conformational restriction. An important additional

advantage of such carbon bridges over the previously summarized restriction are its improved metabolic stability. Especially for small peptides where proteolytic degradation is a major issue, this could improve the bioavailability. Since helical conformations are generally favoured in apolar media, macrocyclization of small, hydrophobic peptide helices has been shown to be well suited to RCM.[10]

In the following chapter an overview of ring-closing metathesis as applied in peptide stabilization and peptidomimetic synthesis is provided. In addition, the biological activity of ring-closed compounds is described.

## *1.2 Beta-turn mimics based on RCM*

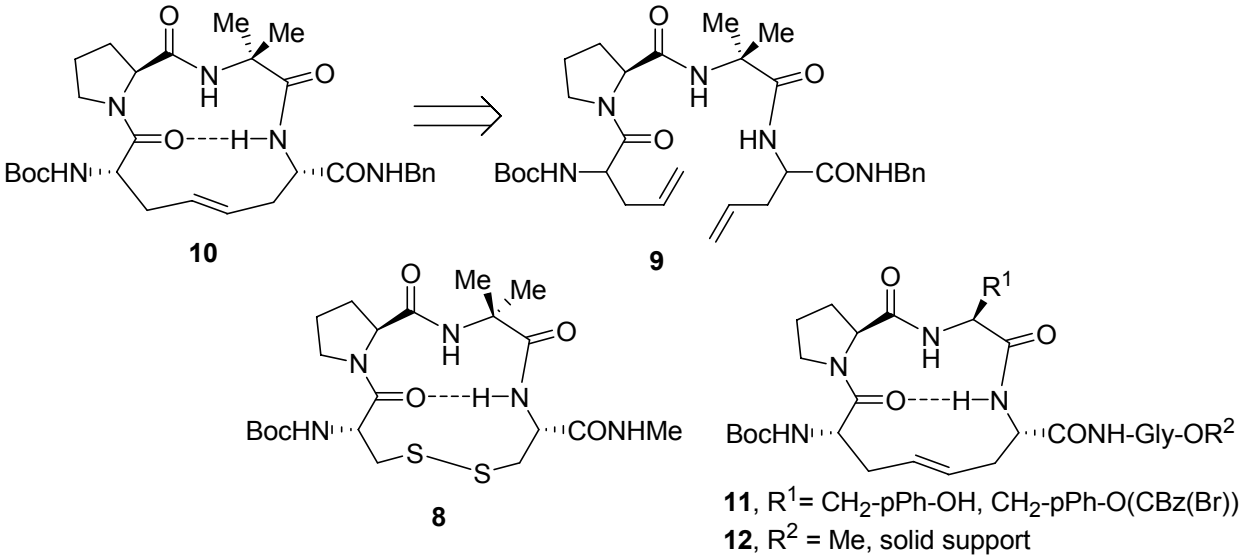
Already in the early stages of the development of olefin metathesis, the Grubbs group used this methodology in peptide synthesis.
Initial studies started with the closure of *N*-allylated allylglycine derivative **2**, giving rise to a six-membered ring. More vigorous conditions were required to close precursors **4** and **6**, which were lower yielding due to the less favorable ring-size of the resulting products.




**Scheme 1.** *Reagents and conditions: a) 1 (5%), PhMe, RT, 2h; b) 1 (16%), PhMe, RT, 24h.*

Grubbs and coworkers also focused on the synthesis of compounds that mimicked natural disulfide-stabilized peptides. As an example, the motif present in **8** is found in redox-active peptides where the sulfur-bridge locks the peptide into a ß-

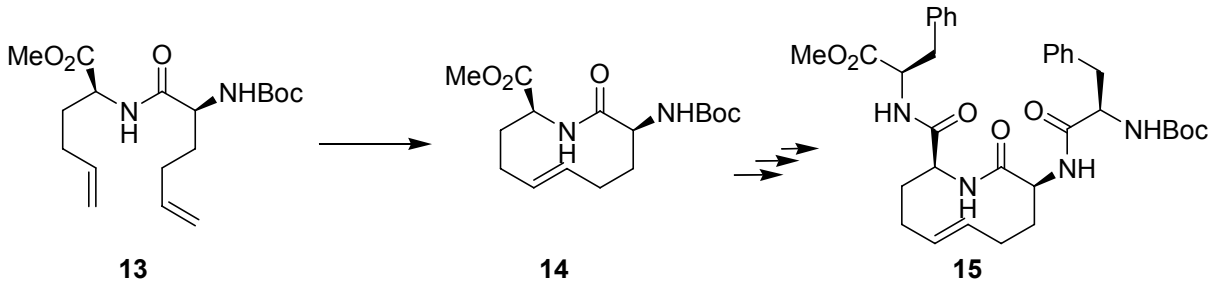
turn.[11] It was thought that replacement of the sulfur-bridge in biologically active compounds by other linkages increases metabolic stability. It was anticipated that the internal hydrogen bond might dispose the diene in a conformation that is well suited for ring closure.




**Scheme 2**. *Balaram's tetramer and Grubbs' carbon mimics.*

Precursor **9** was made using racemic allylglycine residues, but interestingly, instead of forming a statistical mixture of four stereoisomers, only the (*S*,*S*,*S*)-tetramer **10** was formed in 60%. Replacement of 2-aminoisobutyric acid (Aib) by tyrosine (*viz.* **11**),[12] or of the Pro-Aib by the Leu-Leu sequence also produced cyclization products, showing that there is no need for imposing constrained rotation by the *gem*-dimethyl groups (*i.e.* the Thorpe-Ingolg effect) to ensure cyclization. Finally, on solid support (**12**) the reaction also proceeded well.

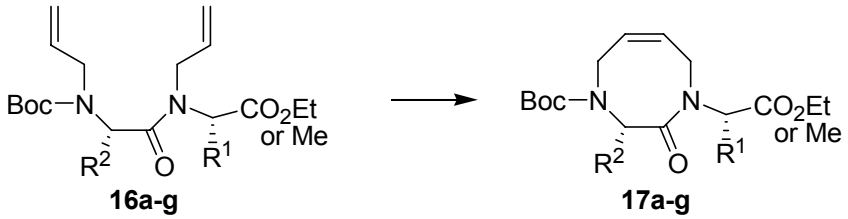
The group of Katzenellenbogen used a different approach to prepare a type I *β*-turn.[13] It was hypothesized that an externally constrained 10-membered ring (Scheme 3) could replace Gly-Leu in Substance P and thus result in increased biological activity.[14] They prepared dipeptide **13**, which was successfully cyclized to ß-turn mimic **14** in 65%. The closure of **13** was the first example of a 10-membered lactam-ring.




**Scheme 3.** *Reagents and conditions:* ***1*** *(10%), $CH_2Cl_2$, RT, 18h (65%)*

With core ring system **14** in hand, **15** was prepared in several steps for conformational analysis and biological evaluation. This analysis showed that the tetrapeptide derivative **15** restricted the central φ and ψ torsion angles to within 30° of the ideal angles for a type I ß-turn.

Further research on small cyclic dipeptides was carried out by Liskamp *et al.* who successfully cyclized *N*-allyl functionalized bulky amino acids.[15] Optimal conditions were found with 1,1,2-trichloroethane as solvent at reflux temperature, using the first generation Grubbs catalyst.

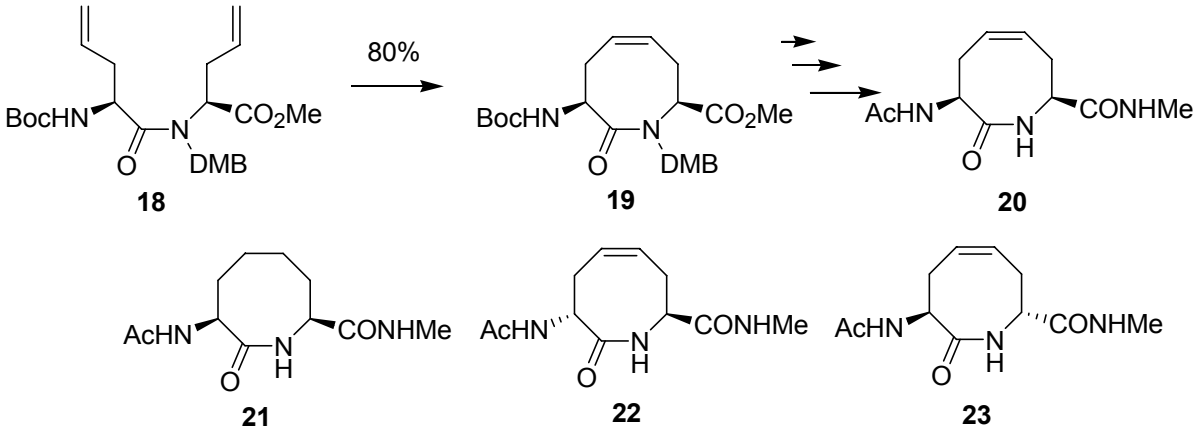



**Scheme 4.** *Reagents and conditions:* ***1****, 1,1,2-trichloroethane, 85-115 °C.*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $R^1$ | H | H | Bn | *i*Bu | H | Bn | *i*Bu |
| $R^2$ | H | Bn | H | H | *i*Bu | Bn | *i*Bu |
| Yield (%) | 73 | 59 | 51 | 50 | 35 | 27 | 53 |

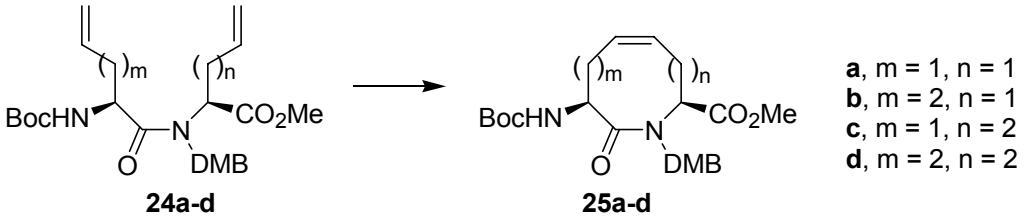
In order to investigate the properties of the ß-turn mimics, the trans-adaptation of secondary amides prevents efficient conversion. *N*-Allyl-substitution masks the properties of the turn but the tertiary structure can adopt a cis-conformation, thus facilitating the ring closure.

Using dimethoxybenzyl (DMB) as a reversible protecting group, Reitz *et al.* successfully cyclized dipeptide **18** to give **19** which was further elaborated into derivatives **20**-**23** of which the ß-turn properties were investigated with X-ray and NMR spectroscopy (Scheme 5).[16] They were found to have a close resemblance to the VIa ß-turn, thus making these unique turns potentially useful tools for novel constrained peptidomimetics.



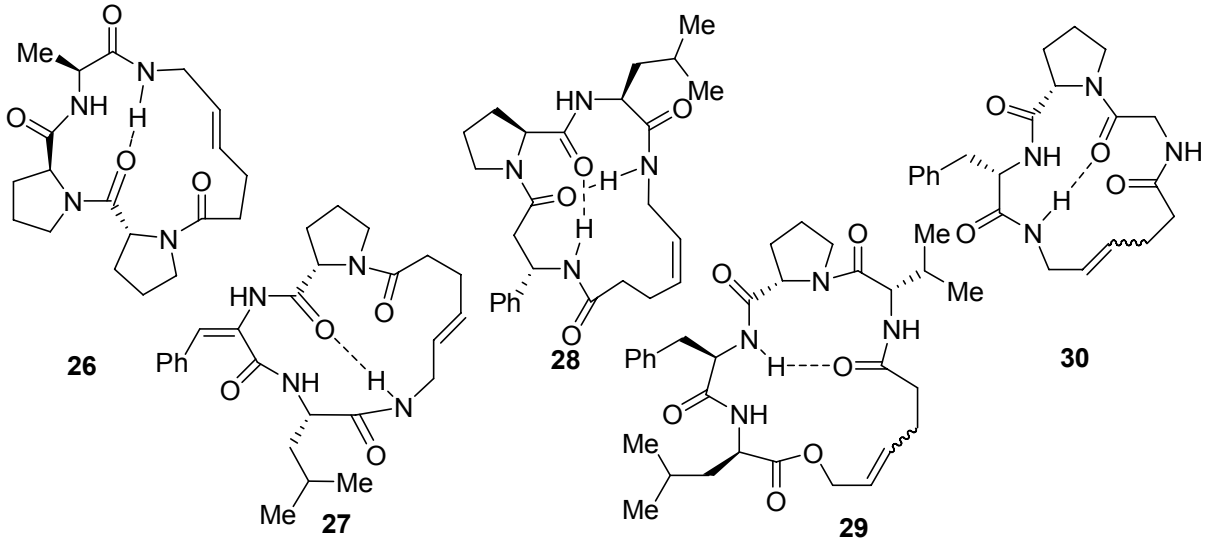

**Scheme 5.** *Reagents and conditions:* ***1*** *(10%), $CH_2Cl_2$, reflux.*

The group of Lubell extended the macrocyclic dipeptide chemistry by making 8-, 9-, and 10-membered rings as shown in Scheme 6.[17] The importance of transient *N*-protection of the central amide is underlined, since only **24d** could be cyclized in unprotected form.




**Scheme 6**. *Reagents and conditions:* ***1*** *(20%), $CH_2Cl_2$, reflux.*

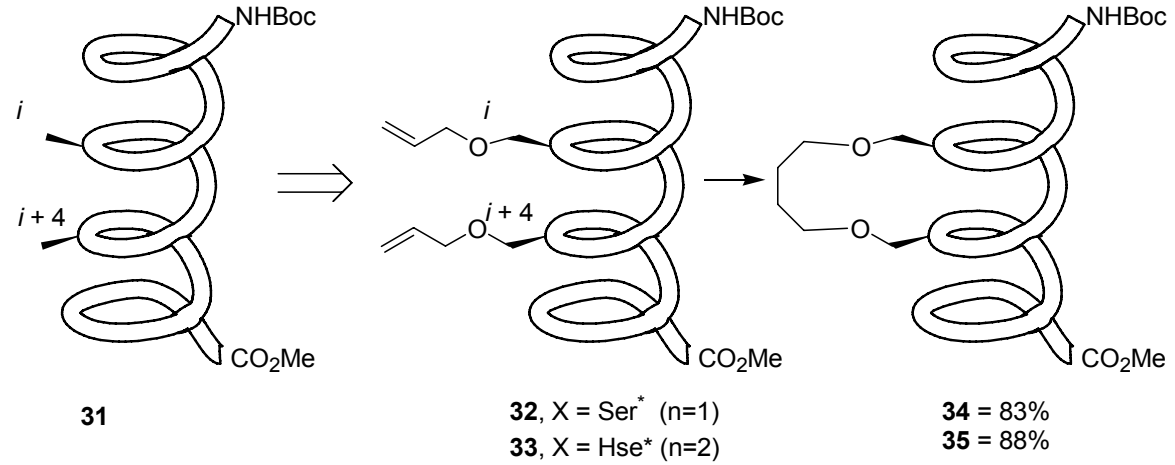
The group of Iqbal used ring-closing metathesis in order to stabilize small cyclic oligopeptides by conformation constrain.[18] After several investigations cyclopeptides **26**-**28** were identified using extensive NMR-studies as constrained $3_{10}$ helical structures (Scheme 7). Also, **29** was found to adopt a type VIa or VIb ß-turn in $CDCl_3$ and the more polar DMSO-$d_6$ respectively.




**Scheme 7**. *Iqbal's cyclopeptides.*

## *1.3 Peptide conformations*

The Grubbs group continued their efforts developing metathesis for carbon-tethering amino acids in a series of helical peptides.[19] The frequently encountered α-helix, as shown schematically as **31** (Scheme 8), fully characterized by Balaram proved to be a good example.[20]

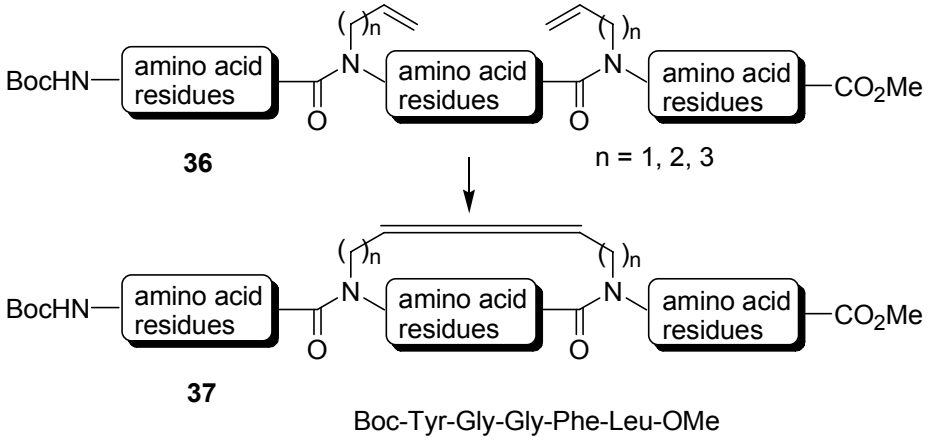



**Scheme 8**. *Reagents and conditions: **1** (20%), $CHCl_3$, RT then $H_2$, Pd/C. (**31** = Boc-Val-**Ala**-Leu-Aib-Val-**Ala**-Leu-OMe, **32**, **33** = Boc-Val-**X**-Leu-Aib-Val-**X**-Leu-OMe) (Hse = homoserine; * = allylated).*

Helical structures **32** and **33** were equipped with allylgroups at the serine positions. After ring-closing metathesis and hydrogenation the peptides **34** and **35** were isolated in excellent yield, which suggests preorganization of the helix. Further research revealed indeed this preorganization of **32** and **33** in apolar solvents.[21] Interestingly, a subtle conformational shift from $3_{10}$ to an α-helix of **34** was observed in $CHCl_3$ as a solvent whereas **35** maintained a $3_{10}$-helical conformation in both solution and the solid state.

Liskamp *et al.* used another approach, coined a 'Rolling Loop Scan'.[22] This phrase involves the functionalization of two variable amides in a peptide with olefinic chains. By having the functionalization at various amide positions the resulting lactam ring size will differ and contain various residues. This should allow a rapid screening of stabilization effects or local conformations of the peptide (Scheme 9). It appeared that some 'rules' apply with respect to orientation of the chain size and successful cyclization. If two amide bonds are involved in the loop, *N*-allylamides have to be used in the starting bis-alkylated peptide (n = 1); if three amides are involved in the loop, *N*-pentenylamides have to be used (n = 3);

if four or more amide bonds are involved in the loop, *N*-butenylamides will work (n = 2).




**Scheme 9**. *Rolling loop scan on Leu-enkephalin (Boc-Tyr-Gly-Gly-Phe-Leu-OMe).*

An important publication of the group of Verdine took an alternative approach by screening multiple configurations of cross-linking where the olefinic chains differed in positions, stereochemistry and length.[23] Incorporating amino acids **38** or **39** in the sequence of RNase A allowed to observe the changes of helical content owing to specific modifications (Figure 3).[24] The peptides were made using solid phase techniques and also ring-closing metathesis was performed on the resin. None of the peptides in the $R_{i,i+4}S(x)$ (Figure 3) series underwent metathesis as the HPLC analysis revealed. Also in the $R_{i,i+4}R(x)$ series only x = 8 went to complete conversion as in the $S_{i,i+4}S(x)$ series x = 7 and x = 8 underwent 68% and >98% conversion. The $R_{i,i+7}S(x)$ series also were more successful with longer chain lengths.

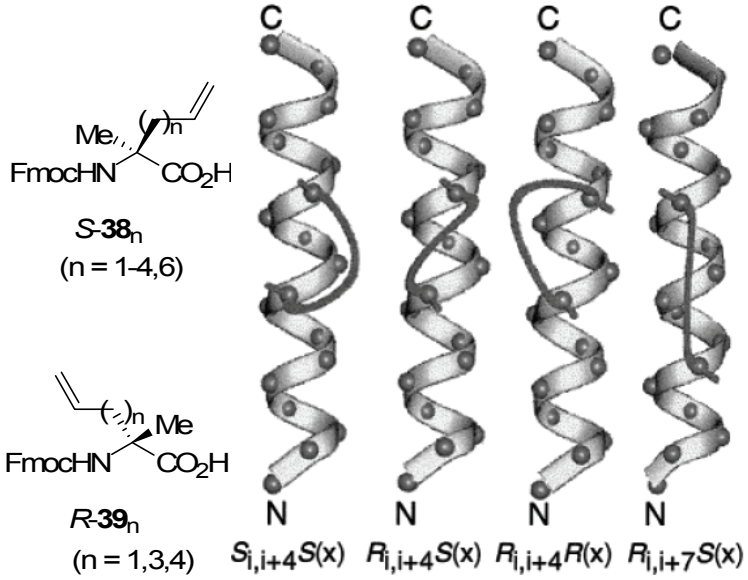
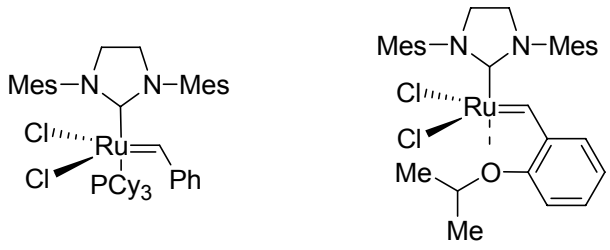



**Figure 3.** *Linked peptides. The nomenclature $R_{i,i+7}S_{(11)}$ refers to a peptide with an R and an S configurated amino acid at positions "i", and "i + 7" respectively, and 11 carbons in the metathesized cross-link.*

Another general trend that was observed is the dramatic effect of ringsize in efficiency of ring-closing metathesis. For example *$R_{i,i+7}S(8)$* underwent no cyclization at all, whereas *$R_{i,i+7}S(9)$* formed its corresponding 31-membered macrocyle to an extent of 51%. This difference might be explained by on-resin preorganization of the α-helix, where the tethers are too short to span the gap.
In order to determine the cross-linking stabilization effect, the helical content of all cyclized peptides were measured quantitatively alongside the natural sequence (Ac-EWAETAAAKFLAAHA-$NH_2$) and the unmetathesized peptide chain.

That conformational restriction does not always lead to the desired results was also found by McNamara *et al.*[25] By incorporation of a carbon-based cystine mimic during pepide synthesis, Ac-Lys-$(Ala)_4$-Lys-$(Ala)_2$-cyclo[Alg-Ala-Lys-Ala-Alg]-$(Ala)_2$-Lys-$NH_2$ was compared to its native 16-mer. Unexpectedly, this artificial bridge did not lead to an α-helical conformation. Instead, depending on the solvent the peptide was found to adopt a ß-turn in either a left-handed or right-handed conformation. It was reasoned that the improper chirality of one of the two stereo centers of the isostere prevents helical formation, just as Verdine found.

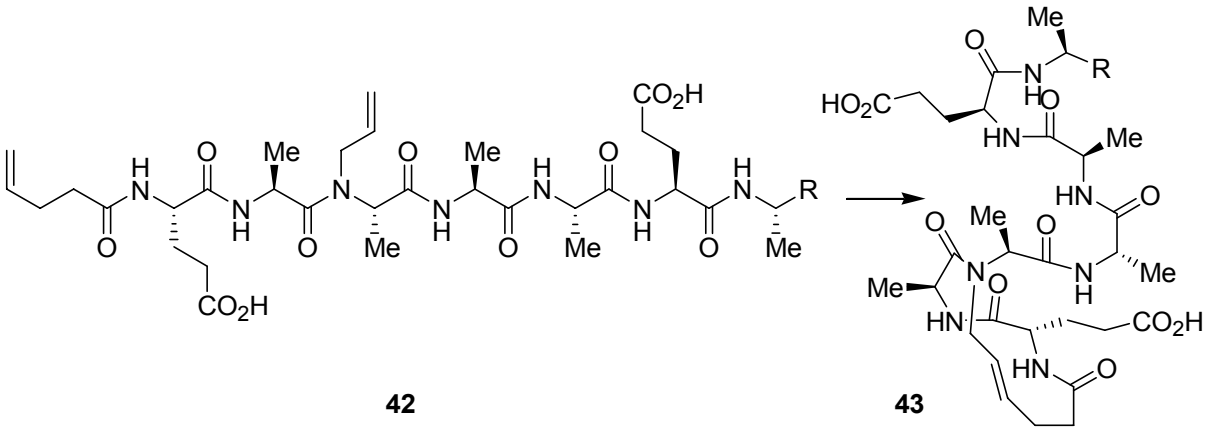
With the discovery of more efficient metathesis catalysts such as **40** and **41** (Figure 4), more research was conducted on peptide systems.




**40** **41**

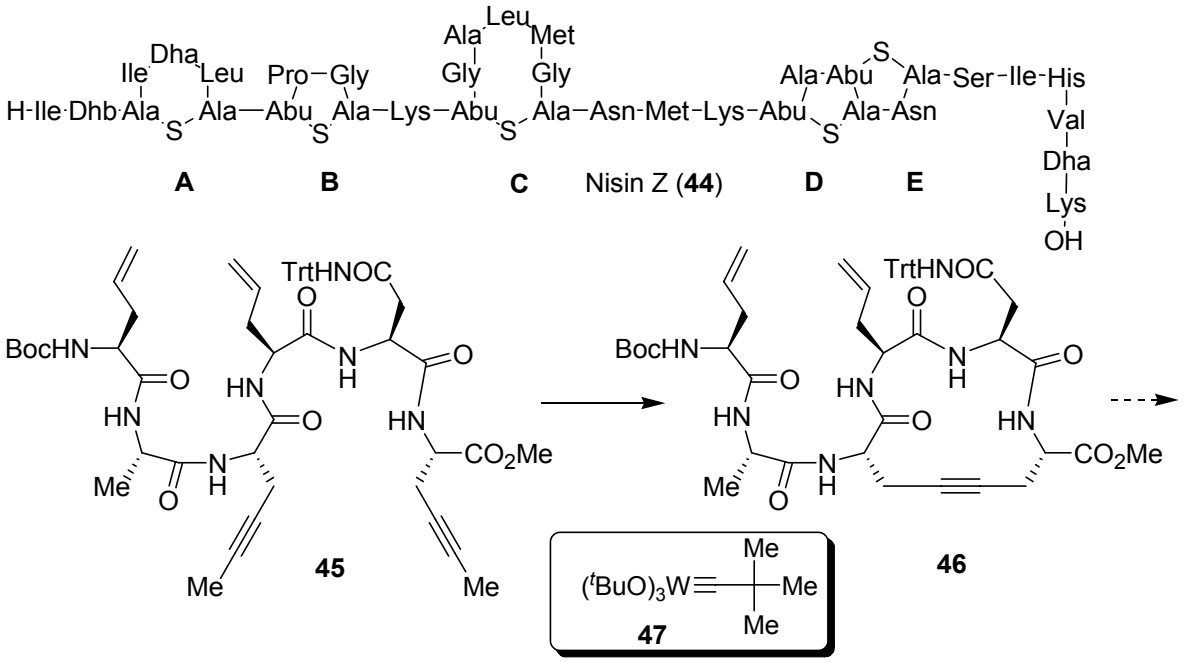
**Figure 4**. *Second generation Grubbs **40** and Hoveyda-Grubbs **41** catalysts.*

The group of Arora investigated the possibility of hydrogen-bond surrogate (HBS) in order to allow full access of natural solvent-exposed surfaces.[26] HBS replaces the hydrogen bond with a carbon-based connection which could artificially constrain an α-helix while maintaining its specific side-chain functionalizations. A small library of peptides sequentially similar to Scheme 10 was successfully cyclized.[27]




**Scheme 10**. *Reagents and conditions:* ***41*** *(10%), 1,2-dichloroethane, 50 °C, 25h (55%).*

Following up on results from our group (see chapter 5),[28] an alkyne-metathesis approach for rigidification was undertaken by Liskamp *et al.*[29] in order to artificially constrain the antimicrobial peptide nisin Z (Scheme 11).[30] This peptide consists of 5 consecutive thioether bridges and the knotted DE-ring at the C-terminus are responsible for its specific biological activity. Besides olefin metathesis they successfully used ring-closing alkyne metathesis (RCAM) to form some cyclic fragments. The advantage is that the triple bond allows selective reduction afterward (see chapter 5).




**Scheme 11.** *Reagents and conditions:* ***47*** *(27%), toluene, reflux (18%).*

An initial approach involving a suitably configured peptide with four olefin metathesis handles (resembling peptide **45**) failed. In contrast, peptide **45** containing two alkyne functionalities was made and RCAM was successfully

applied. The second ring-closure of **46** was unfortunately due to solubility issues impossible. However, this represents a nice example where RCM and RCAM can be successfully used alongside each other without interference for the specific construction of different carbon bridges.

## *1.4 Facilitating cyclizations*

Noting the problems associated with difficult on-resin preorganization Kessler and coworkers[31] attempted to rigidify peptides by introduction of Mutters pseudoproline Ser($\psi^{Me,Me}$pro) moiety.[32] Comparing the conversion rates of RCM of the rigidified 10-mer with those of the unmodified molecule (serine instead of pseudoproline), the cyclization proceeded smoothly with excellent conversion rates (Figure 5).

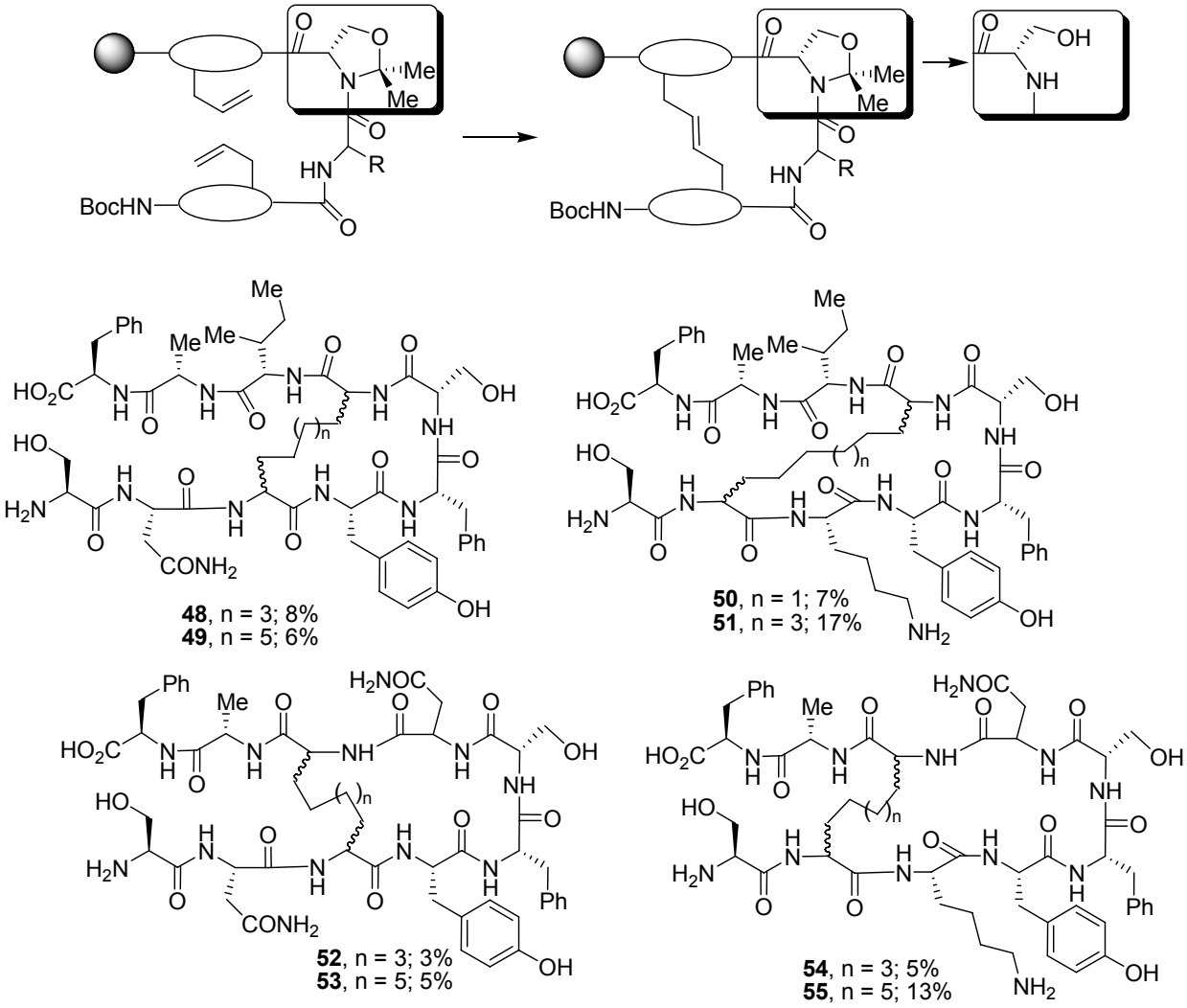
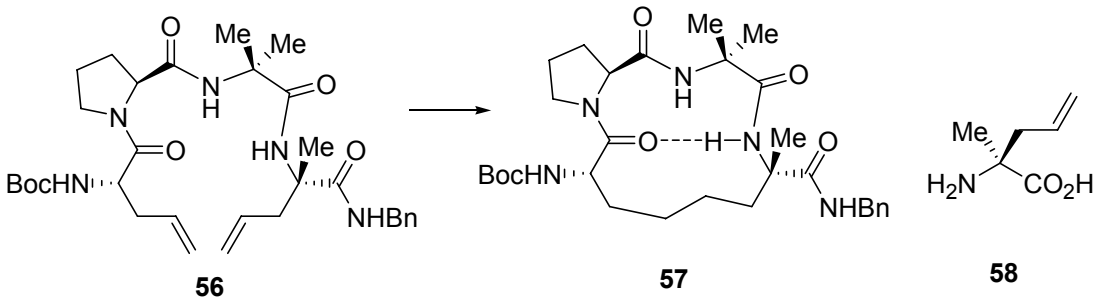



**Figure 5**. *Peptides constructed using the Ser($\psi^{Me,Me}$pro)-motif. Each set consists of approximately equimolar mixtures of the four stereoisomers with varying ring sizes. Yields are for reduction, deprotection and purification using RP-HPLC.*

The introduction of pseudoproline might facilitate the metathesis reaction of 10-mer peptides, but an obvious drawback is the presence of serine at a specific position.

The observed difficulty of several cyclizations and the preferential preorganization was also observed by Toniolo and coworkers.[33] They applied $C^{\alpha}$-methylated allylglycine (**58**) and its turn/helix forming properties in ring-closing metathesis of peptide **56** and compared the properties of the resulting cyclic peptide **57** with Grubbs' cyclopeptide **10** and Balaram's cyclic derivative **8**. NMR-studies indicated a markedly increase in intramolecular H-bonding.




**Scheme 12**. *Reagents and conditions:* ***40****, PhMe, 16 h, 80 °C then* $H_2$*,* $PtO_2$ *(85%).*

# *1.5 Peptidomimetics*

Particular applications of contrained peptides are found in the field of peptidomimetics. Signal transduction proteins play a major role in the complex dynamic networks that regulate cell function and are intensively studied for their potential as drug targets. Much attention is being paid to the (mammalian) Grb2 SH2 domain which consists of an SH2 domain flanked by two SH3 domains. This key component of the Ras signaling[34] pathway is an important regulator of cell growth and differentiation and therefore a potential drugs target for cancer therapy.[35]

Binding of natural phosphorylated tyrosine-containing ligands of Grb2 SH2 domains take place in type-1 ß-bend fashion.[36] The sequence -pTyr-Val-Asn-Val- is recognized by the Grb2 SH2 domain and specifically the asparagine is found to be very important.[37] Based on this motif, inhibiters were designed to mimic this particular sequence.

Caravatti[38] and Ettmayer[39] explored the usage of cyclic peptides to serve this purpose. A cystine containing pentamer of Caravatti (Ac-Cys-pTyr-Ile-Asn-Cys-$NH_2$ (**59**)) was found to be the most active one ($IC_{50}$ = 0.37 µM) of a small

library.[40] Ettmayer confirmed the ß-hairpin recognition by the SH2 domain and demonstrated that the stabilization increased the potency.

Based on these findings, the groups of Burke[41] and Liskamp[42] intended to apply ring-closing metathesis to prepare mimics by constructing a constrained carbon backbone for SH2 inhibition. Burke used **60** as model platform and successfully prepared the cyclic mimic **61**.

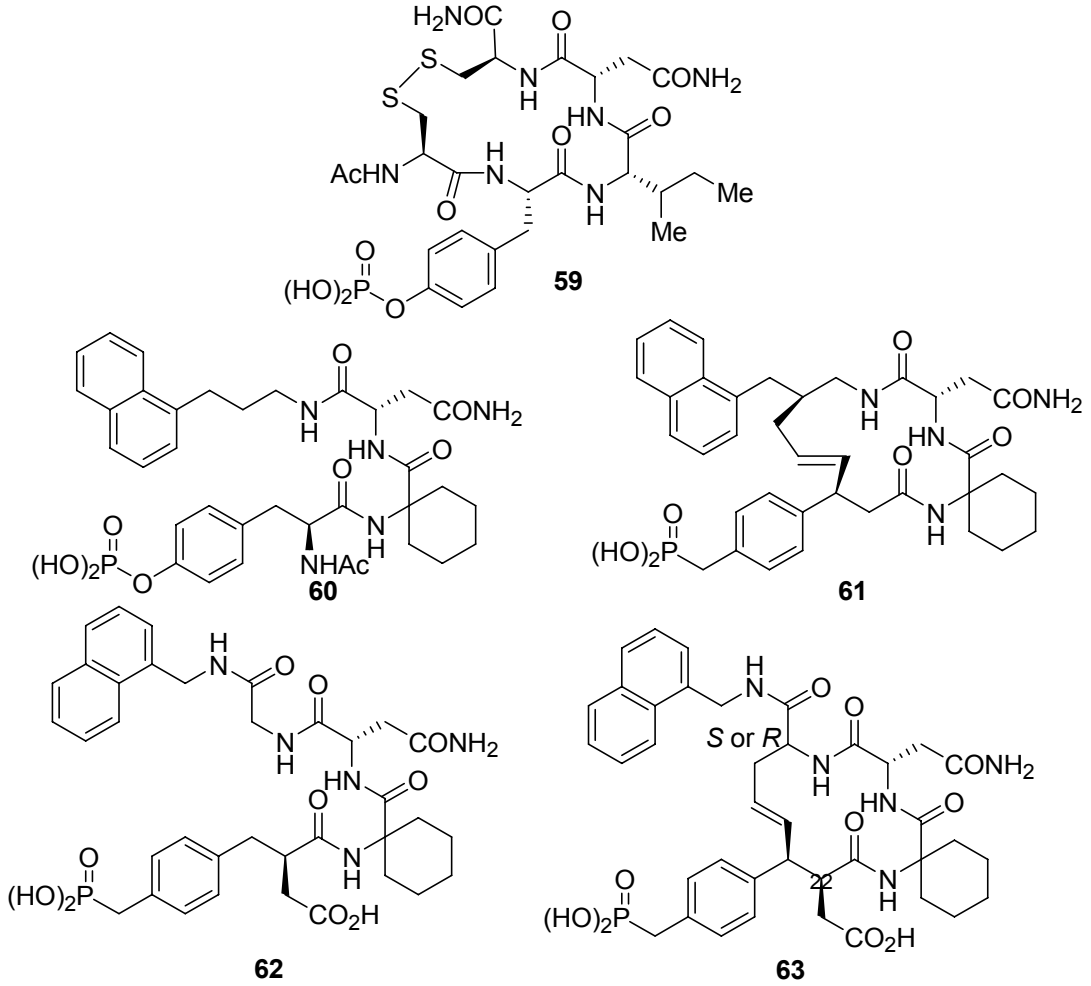



**Figure 6**. *Burke's Grb2 SH2 inhibitors.*

The increase of stability led to a 100-fold improved activity ($IC_{50}$ from 2 µM to 0.02 µM) over its unconstrained form but the initial compound **60** had, due to the presence of the acetate group also a similar activity ($IC_{50}$ = 0.02 µM). Burke then redesigned the mimic in order to include the functional group at position 22 and found for (*R*)-**63** ($K_d$ = 54.9 nM and (*S*)-**63** ($K_d$ = 22.7 nM) an enhancement of two orders of magnitude compared to unstabilized **62** ($K_d$ = 5610 nM).[43]

Liskamp *et al.* also prepared a cyclic phosphopeptide for modulation of the signal transduction *via* the Grb2 SH2 domain. The use of RCM on an alternative sequence (Figure 7) was anticipated to provide the proper conformation for

optimal interaction with the SH2 domain. However, after cyclization the additional constraint of **65** did not lead to improved biological activity ($K_d$ = 440 nM to $K_d$ = 600 nM). This result shows that covalent control of the peptide conformation is a subtle process in which other factors may play a dominant role as well.

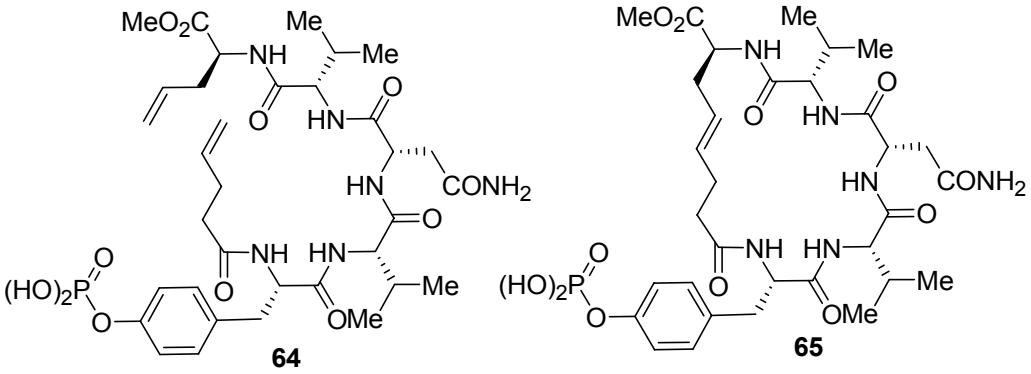



**Figure 7**. *Liskamp's Grb2 SH2 inhibitors.*

The group of Liskamp also demonstrated the difficulties of peptide RCM in the synthesis of an MC4-receptor based on cyclopeptide **66**.[44,45] On resin cyclization was unsuccessful and selective cleavage resulted in mostly insoluble peptide. After using co-solvents, compound **67** was obtained but biological activity remained in the same order of magnitude as its linear starting material ($EC_{50}$ = 0.21 nm and 0.34 nM, respectively (**66** = 0.01 nM)).

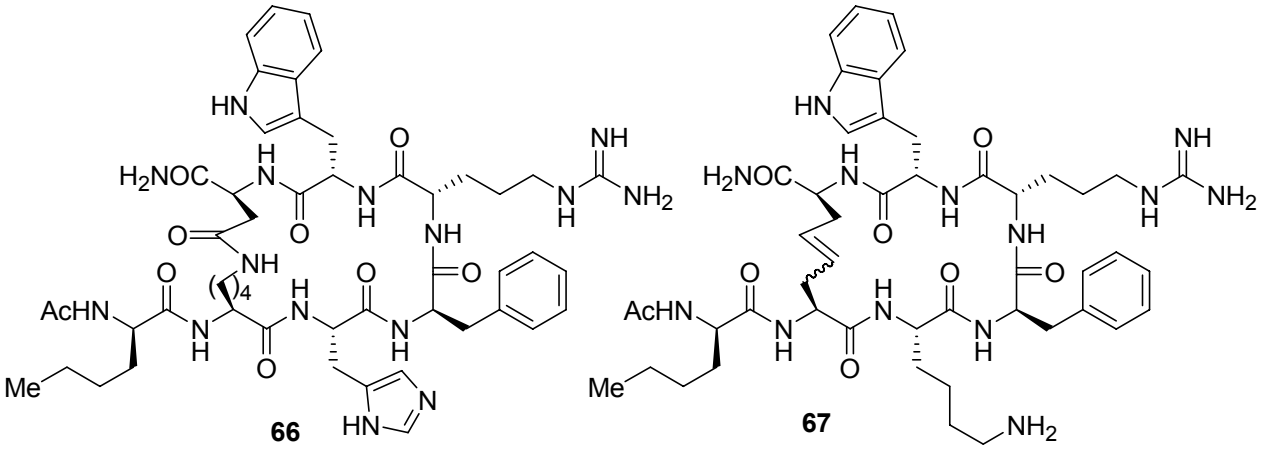



**Figure 8**. *MC4 receptor **66** and Liskamp's MC4 receptor **67**.*

The Coates group[46] investigated the biological activity of carbon mimics of the disulfide-constrained Bowman-Birk inhibitor.[47] Using microwave assistance, several (*R*)-allylglycine-containing peptides were successfully cyclized, but their biological activity was at least a 10-fold less than their (*S*) counterparts. In

summary, the 31-membered macrocycle **71** showed competitive inhibition-values compared to disulfide **68** (Figure 9).

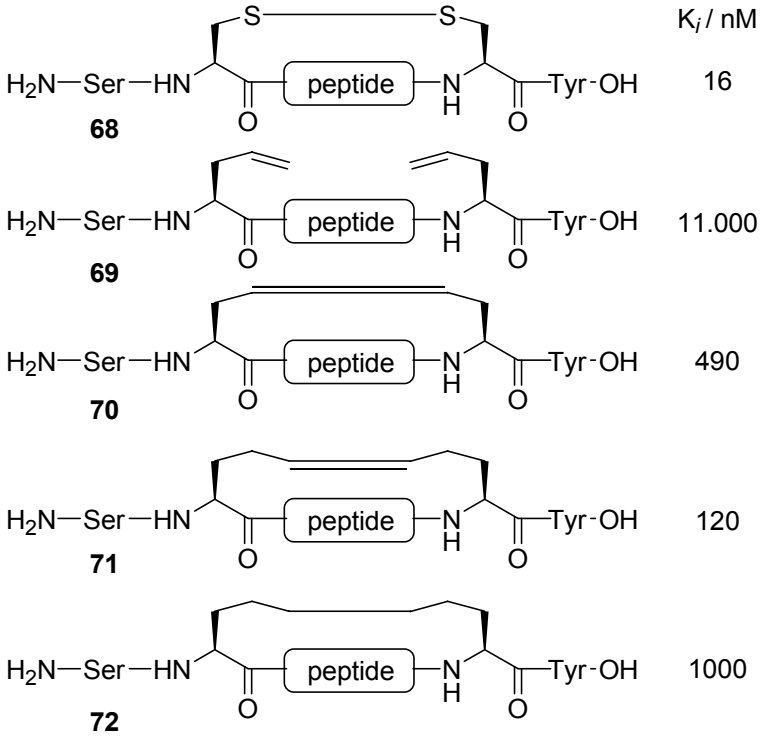



**Figure 9**. *Coates series of Bowman-Birke inhibitors*

Carbon analogues of oxytocin[48] (**73**) have been reported by Vederas *et al.*[49] In this example the biological activity of native oxytocin ($EC_{50}$ = 2.7 ng/mL) was diminished by the introduction of the carbon bridge to 38 and 242 ng/mL for the cis- and trans-analogues of **74** respectively. Hydrogenation of the mixture to the corresponding saturated system reduced the activity to 338 ng/mL.

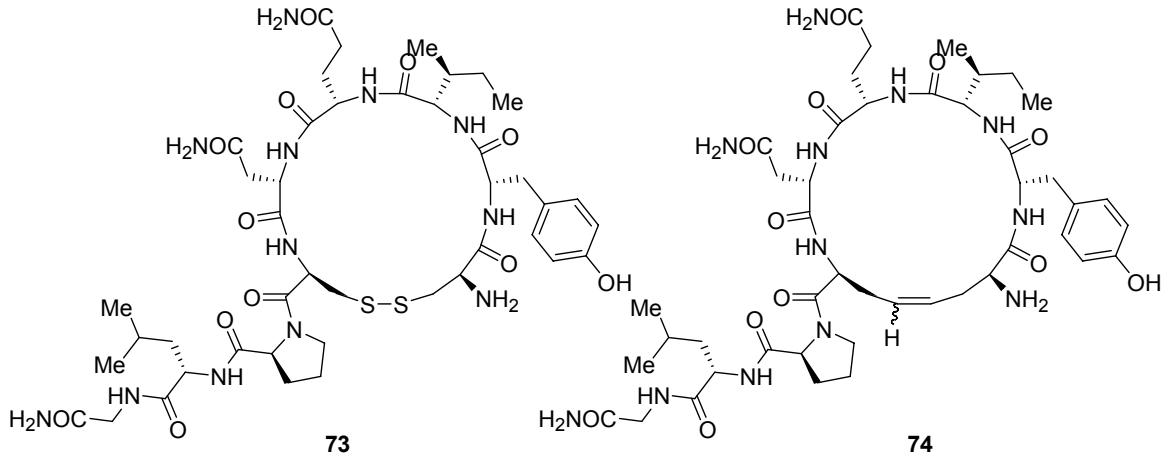



**Figure 10**. *Oxytocin (**73**) and carbon analogues (**74**).*

## 1.6 Conclusions

Ring-closing metathesis stands out as a powerful method to introduce conformational restriction. On the one hand, the tolerance of the catalyst with respect to the peptide functional groups and on the other hand the enhanced metabolic stability of the carbon analogues, provides interesting opportunities. However, simple replacement of cystine by carbon analogues does not automatically lead to enhanced biological activity. The locking of peptide motifs requires the peptide to preorganize in order to undergo successful ring-closing metathesis, which may be reached by temporary *N*-functionalization of the backbone to enhance its reactivity.
Despite the unpredictability of ease of cyclization and of the enhanced biological activity, ring-closing metathesis is certainly an useful tool for conformational restriction.

## 1.7 Purpose and outline of this investigation

In this thesis, the application of new methods to introduce conformational restriction in peptides is investigated.
In chapter 1, an overview is given of recent applications of ring-closing metathesis being the commonly applied method to introduce conformational restriction in peptides. This demonstrates the potential of methylene linked restrictions in understanding peptide conformation and enhancing biologically active of peptidomimetics.
In chapter 2 details are given concerning optimized chemo-enzymatic strategies for the required non-proteinogenic unsaturated amino acids.
Chapter 3 details synthetic investigations into the application of zinc/copper-mediated couplings to functionalize acetylenic amino acids with alkyl-groups.
In chapter 4 preliminary results are given detailing attempts to use Cu(I) catalyzed cycloadditions for restriction of peptides.
Chapter 5 ring-closing alkyne metathesis is introduced as a new tool to generate conformational restriction in peptides.
In chapter 6 a natural product synthesis is pursued in order to compare the synthesis and activity of FE399 and its carbon-derivative.
This research was conducted as part of a STW-collaboration. The other members of this project were Prof. Hans Schoemaker (University of Amsterdam/DSM research), Prof. Jan van Hest and Dr. Lee Ayres (Radboud University Nijmegen)

and Prof. Herman Overkleeft, Dr. Mark Overhand and Dr. Gijs Grotenbreg (University of Leiden).

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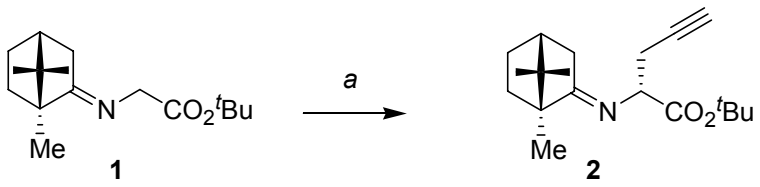
# 2 SYNTHESIS OF ENANTIOPURE ACETYLENE-CONTAINING AMINO ACIDS

## *2.1 Introduction*

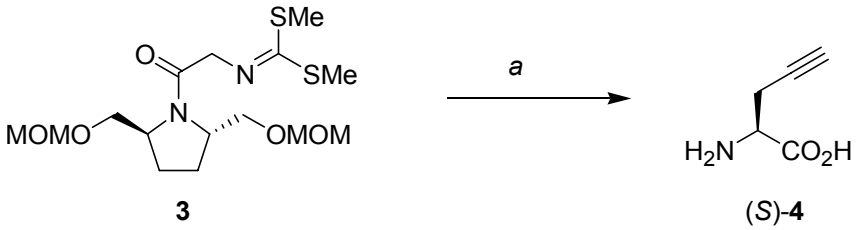
Acetylene-containing amino acids play an increasingly important role as witnessed by the numerous applications in synthesis and chemical biology.[1] Throughout this thesis several facets of the versatility of these amino acids are shown. Since the availability of these compounds in enantiopure form is crucial, this chapter will focus on enantioselective syntheses of these building blocks. Many synthetic strategies have been developed over the years and a brief overview of representative methods, each with its own distinct advantages and disadvantages, will be provided.[2]

Most of the initial examples rely on diastereoselective alkylations employing suitable chiral auxiliaries. An early example starts from imine **1**, derived from (*R*)-camphor and glycine *tert*-butyl ester, which was alkylated by Chadha and coworkers in a moderately diastereoselective manner (Scheme 1).[3]



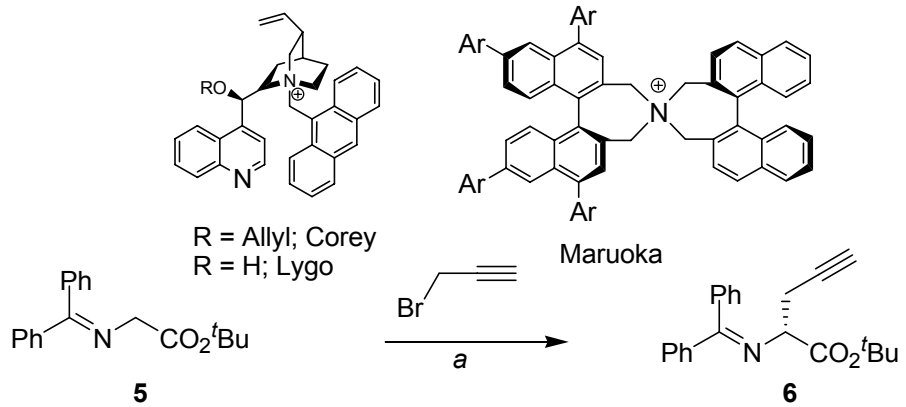

**Scheme 1**. *Reagents and conditions: a) LDA, propargyl bromide, THF, 78%, 67% de.*

Inversely, introduction of a chiral ester auxiliary may lead to optically active amino acids via a similar alkylation procedure.[4] The example shown in Scheme 2 shows an excellent diastereoselective alkylation (98% de), albeit that in this case the method is less attractive due to a troublesome removal of the chiral auxiliary thereby reducing the overall yield to 51%.




**Scheme 2**. *Reagents and conditions: a) LDA, propargyl bromide, THF then HCl, THF/$H_2O$, 51%, 98% ee.*

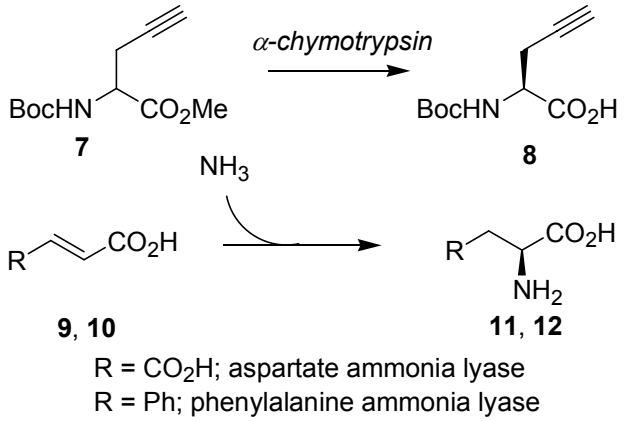
Since then, several examples of *N*-tethered chiral auxiliaries have emerged derived from sulfonamides,[5] amino acids or peptide-based nickel complexes.[6,7]
In contrast to alkylation reactions where a stoichiometric amount of the chiral auxiliary is required, more recently attention has been paid to enantiomerically pure phase-transfer catalysts (Scheme 3) that provide the desired amino acid using a catalytic amount of the chiral reagent in a two phase system. After the two phase protocol for the production of racemic amino acids had already been developed some time ago by O'Donnell,[8] the Corey group and Lygo's group showed almost simultaneously that use of cinchonidine- or cinchonine-derived chiral phase-transfer catalysts gave the corresponding amino acids in good to excellent ee's. Both methods differ only in the functionalization of the hydroxyl group of the chiral ligand.[9,10] A completely different class of BINAP-based catalysts was recently introduced by Maruoka,[11] who showed that they can be used to give the alkylation products in high optical purities (up to 98%) with rather low catalyst loadings of 1 mol%. The efficiency of this elegant method can be enhanced by the addition of crown ethers.[12]




**Scheme 3**. *Reagents and conditions: a) PhMe, 50% aqueous KOH, 0 °C, 92%, 86% ee (Ar = 3,5-diphenylphenyl).*

Apart from chemical methods, biocatalysts have since long also been applied for the preparation of acetylene-containing amino acids. As early as in 1969,

Havinga demonstrated that propargylglycine could be obtained in enantiopure form using a lipase.[13] In the late 1980s, Whitesides showed that acylases could be used for the synthesis of enantiopure propargylglycine.[14] More recently other enzymatic methods followed, for example involving lipases.[15] One of the more recent examples was published by the group of Nosal, who employed the enzyme α-chymotrypsin to resolve propargylglycine derivative **7**.[16] As in this case, most of these enzymatic reactions lead to the availability of both enantiomers, which may be an advantage. Other specific biocatalysts can be deployed for direct selective amination or alkylation,[17] giving rise to only one enantiomer altogether as exemplified for **11** and **12**. However, no specific enzymatic system has been published to prepare propargylglycine in this way.



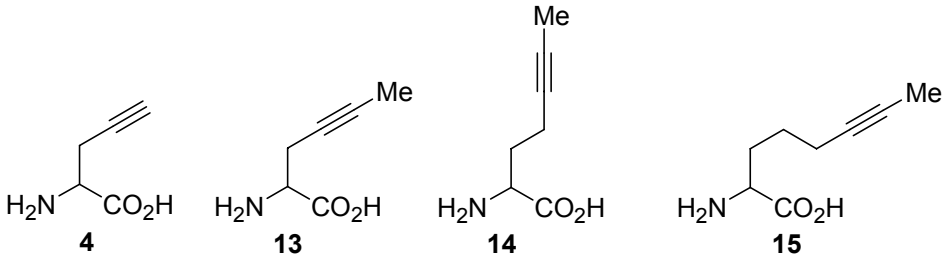

**Scheme 4**. *α-Chymotrypsin, 0.1 M phosphate buffer pH = 8, 20 h, ee >99%.*

The high selectivity that is often displayed by enzymes, combined with the fact that the production of enzymes is easy to scale up, makes that enzymatic resolution can be readily employed for industrial purposes. Nowadays, various of such industrial scale syntheses exist, each of them responsible for the production of several thousands of tons of enantiopure amino acids.[18]

In conjunction with a chemoenzymatic pathway that was developed in our group in collaboration with DSM (Geleen, The Netherlands) for the synthesis of acetylene-containing amino acids,[21] this chapter will detail the optimization of some of our previous routes and thus show how the amino acids employed in this thesis were obtained. These amino acids will be used for several applications as described in the additional chapters of this thesis.

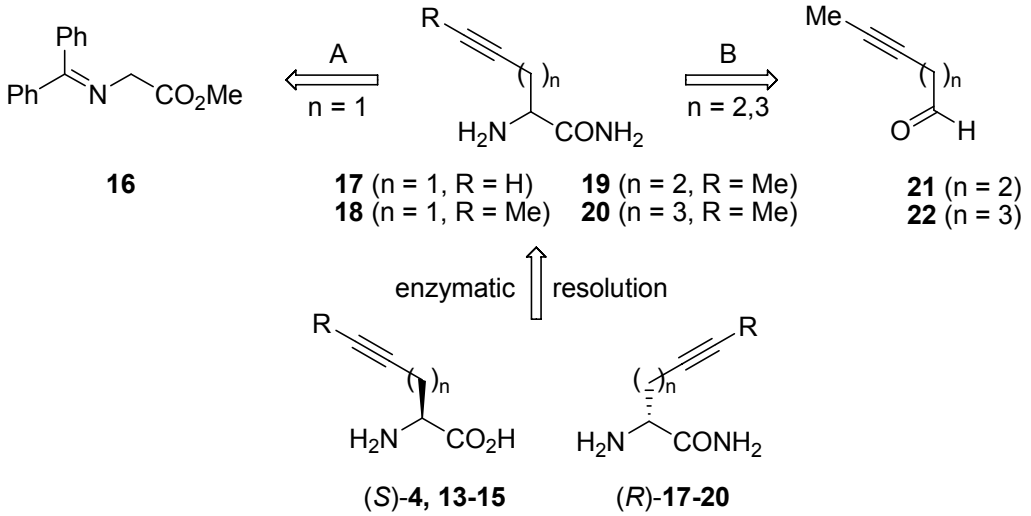
# *2.2 Retrosynthetic approach*

The focus of this chapter will be on the synthesis of the non-proteinogenic amino acids **4** and **13-15** (Scheme 5) in both enantiomeric forms. [1,19,20,21]




**Scheme 5.** *Acetylene-containing amino acids, used in both enantiomeric forms in this thesis.*

The retrosynthetic approach is shown in Scheme 6. Key in this process is an enzymatic resolution of the racemic amino acid amides **17-20**, which are enantioselectively hydrolyzed by an aminopeptidase to the give the (*S*)-acids **4**, **13-15**, while the (*R*)-amides **17-20** remain intact.



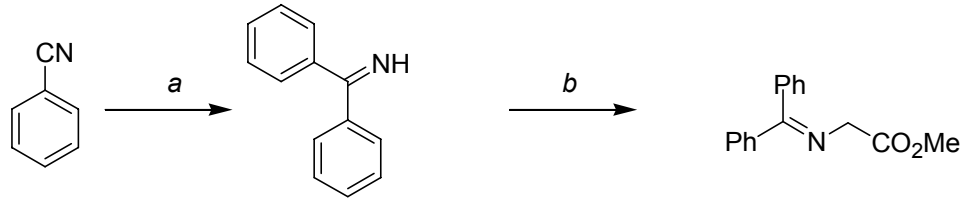

**Scheme 6**. *Retrosynthetic approach for the synthesis of the acetylene-containing amino acids.*

The required amino acid amides **17-20**, in turn, can be obtained in several ways. In our group, we mainly relied on two approaches being (i) alkylation of the so-called O'Donnell synthon **16** (Scheme 6, path A),[22] and (ii) a modified version of the Strecker reaction[23] (path B).[24] The latter pathway, requiring the corresponding aldehydes **21** and **22** as starting materials, generally has a higher atom efficiency. However, a drawback of the latter method is that allylic and propargylic amino acid amides (n = 1) cannot be made in this way. This is due to the required ß,γ-unsaturated aldehyde which is rather unstable due to facile isomerization and/or enolization followed by subsequent aldol processes.

Therefore, the O'Donnell alkylation pathway was chosen for the propargylic amino acid amide derivatives.

## *2.3 The O'Donnell approach (path A)*

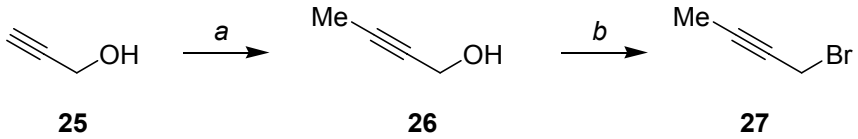
The O'Donnell synthon **16** can be conveniently prepared on mol scale following a literature procedure involving a reaction of benzonitrile with phenylmagnesium bromide,[25] followed by transimination with the HCl-salt of glycine methyl ester.




**Scheme 6**, *Reagents and conditions: a) PhMgBr, $Et_2O$, 40 °C (73%); b) HCl·$H_2N$-Gly-OMe, $CH_2Cl_2$ (93%).*

A particular advantage of ketimine **16** is the fact that the bulky benzophenone substituent prevents the formation of dialkylated products. Furthermore, the ketimine can be efficiently hydrolyzed in a later stage under mildly acidic conditions, which do not affect the acetylene functions.

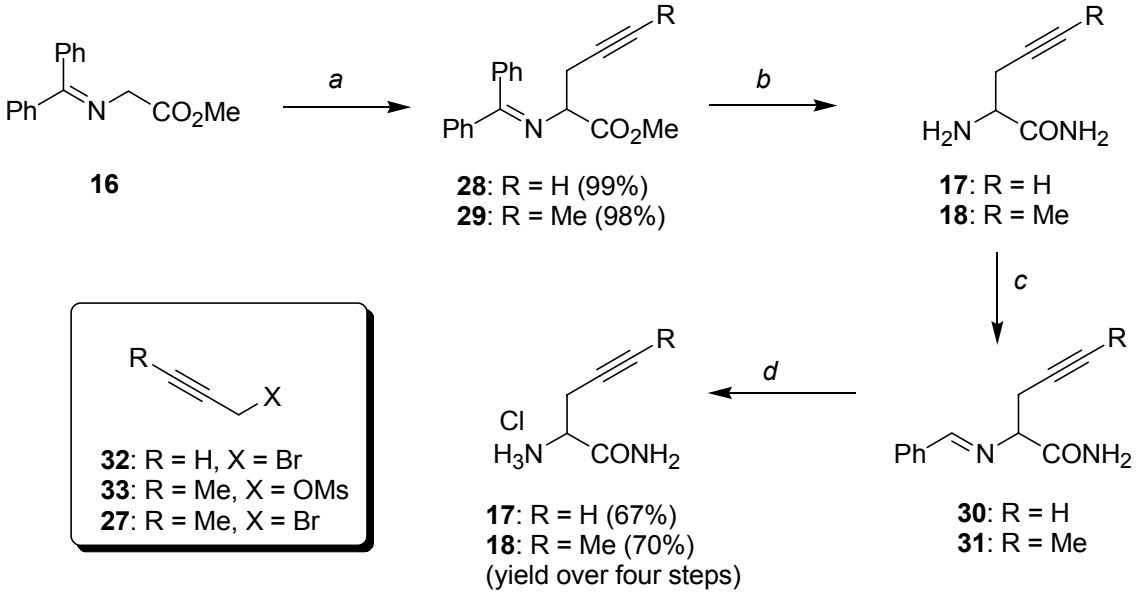
Synthesis of the propargylic amino acids **4** and **13** requires alkylation of the O'Donnell synthon **16** with propargyl bromide (**32**) and 1-bromo-2-butyne (**27**), respectively. While propargyl bromide is relatively cheap, its methylated counterpart **27** is rather expensive. Therefore, preparation of the latter compound from inexpensive propargyl alcohol (**25**) is more cost effective, especially for large scale synthesis as in our case. Thus, propargyl alcohol was deprotonated twice using lithium amide in liquid ammonia (the dilithium-salt precipitates completely in ammonia), followed by addition of 1 equiv of methyl iodide to give the monomethylated product 2-butynol (**26**). This was then transformed into the corresponding bromide **27** using 0.35 equiv of phosphorous tribromide (Scheme 7). Careful addition of alcohol **26** to the tribromide at –78 °C resulted after several hours in the corresponding phosphite, which upon refluxing was converted into the desired bromide **27**.




**25** **26** **27**

**Scheme 7.** *Reagents and conditions: a) $LiNH_2$,, $NH_3$, –33 °C, then MeI; b) $PBr_3$, $Et_2O$, –78 °C to reflux (47%, yield over 2 steps).*

Alkylation of the O'Donnell synthon **16** was performed using sodium hydride (1.1 equiv), the halides **27** and **32** (1.3 equiv) and a catalytic amount of lithium iodide in refluxing THF. The reactions were monitored by TLC and stopped directly upon completion in order to suppress degradation of the product. Besides the use of bromide **27**, the alkylation was also carried out with the corresponding mesylate **33**, prepared from **26** upon reaction with mesyl chloride. However, this resulted in a rather poor yield of 45%.



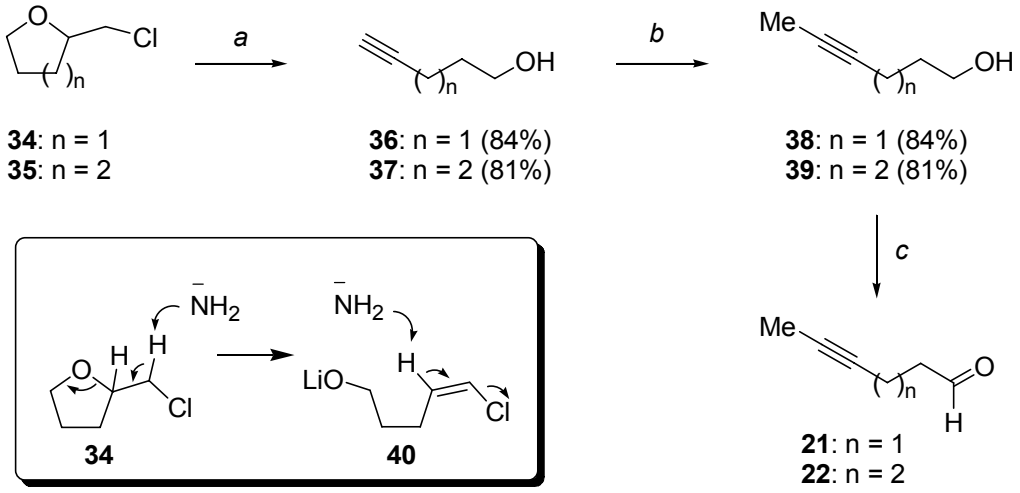

**Scheme 8**. *Reagents and conditions: a) **27** or **32**, NaH, LiI, THF, 60 °C; b) i: 1 M HCl, $Et_2O$, ii: $NH_4OH$; c) benzaldehyde, NaOH; d) concentrated HCl, acetone.*

Using bromides **27** and **32** on the other hand, the alkylated imines **28** and **29** were obtained in excellent yield. Treatment of the crude reaction mixture with 1 M HCl in a two phase system with ether released the free amine, after which the ester was reacted with concentrated ammonia (25% in water) to yield the required amides **17** and **18**. During this conversion, partial hydrolysis[26] of the ester was observed so that separation of the acid from the amide was necessary. This could be efficiently accomplished via treatment of the mixture with benzaldehyde under basic conditions. The amide selectively reacted with benzaldehyde to form the corresponding Schiff base (**30**, **31**), so that the resulting mixture could be partitioned between the water soluble amino acid and the water

insoluble Schiff base. Redissolving the Schiff base in acetone and treatment with equimolar concentrated hydrochloric acid led to hydrolysis of the imine function and precipitation of the amino acid amide as its HCl-salt. Simple filtration then yielded the desired amides **17** and **18** in good overall yields of 67 and 70%, respectively. Due to the fact that the products can be readily purified in the final step, the whole sequence can be carried out without intermediate chromatography and therefore easily scaled up.

## *2.4 Strecker synthesis (path B)*

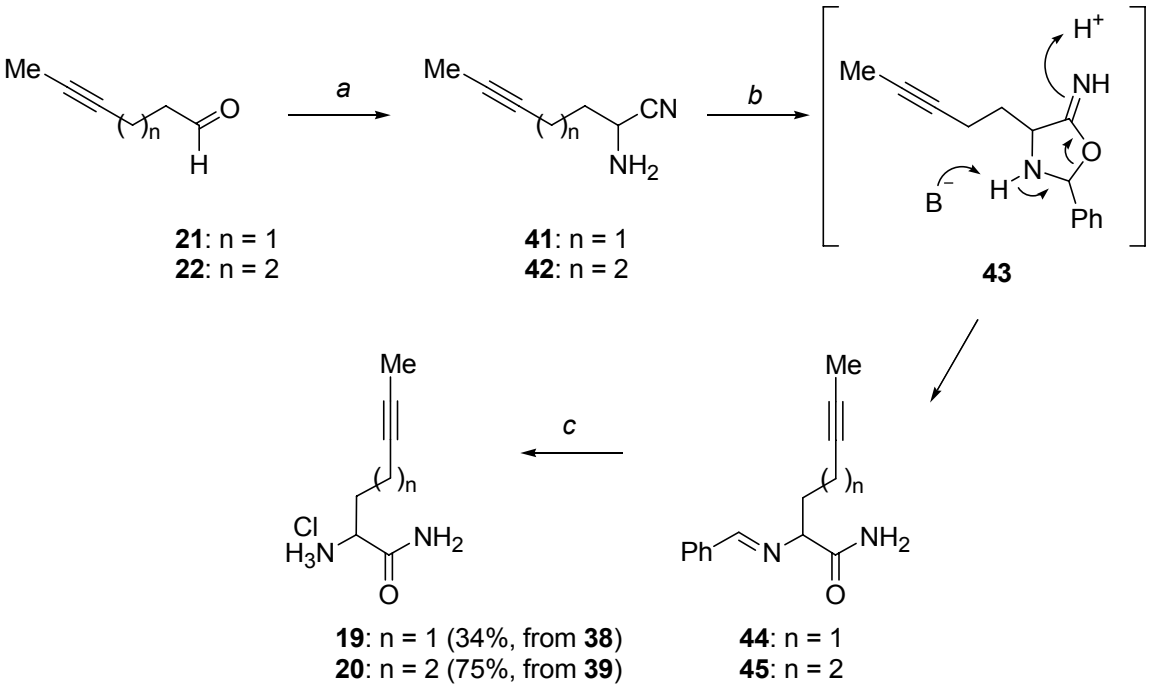
In the Strecker reaction, an aldehyde is transformed into the corresponding aminonitrile, which in turn can be hydrolyzed into racemic amino acids in high yields. In Scheme 10, a modified pathway is shown which leads to the required amino acid amides. This involves the availability of aldehydes **21** and **22**, which were prepared on large scale as shown in Scheme 9. Commercially available 2-chloromethyltetrahydrofuran (**34**) and 2-chloromethyltetrahydropyran (**35**) were both treated with sodium amide in liquid ammonia to undergo a double elimination, thus affording the acetylenes **36** and **37**. Subjection of the crude acetylenes to lithium amide in liquid ammonia, followed by the addition of one equiv of methyl iodide provided the alcohols **38** and **39**, which were obtained in good overall yields after distillation.[27] Subsequently, the alcohols were oxidized to the corresponding aldehydes **21** and **22** using a typical Swern oxidation.




**Scheme 9**. *Reagents and conditions: a) $NaNH_2$, $NH_3$; b) $LiNH_2$, $NH_3$, then MeI; c) $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$, –78 °C.*

The aldehydes **21** and **22** were in crude form directly subjected to the modified Strecker reaction. This reaction commenced with the condensation of ammonia

with aldehydes **21** and **22**, followed by addition of *in situ* generated hydrogen cyanide (from equimolar amounts of $NH_4Cl$ and KCN). This led to the aminonitriles **41** and **42**, which after evaporation were immediately treated with benzaldehyde and NaOH at pH = 10. This led to conversion of the amino function into the Schiff base with concomitant partial hydrolysis of the cyanide group. This process presumably proceeds in an intramolecular fashion through the intermediate five-membered ring *N*,*O*-acetal **43** as shown in Scheme 10. [28]



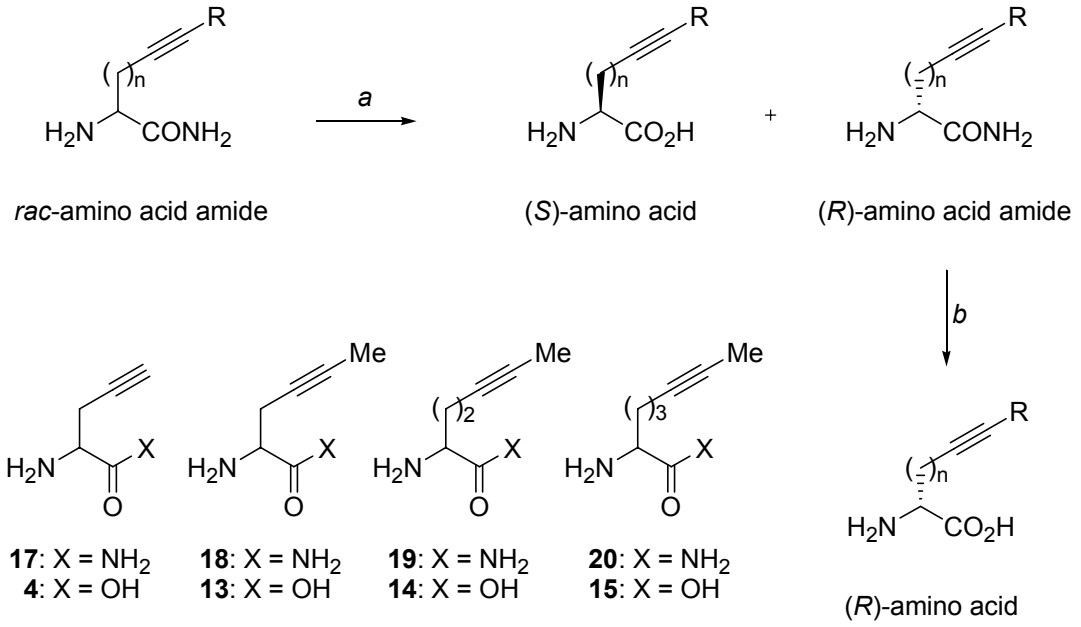

**Scheme 10**. *Reagents and conditions: a) NaCN, $NH_4Cl$, $NH_4OH$, $H_2O$; b) PhCHO, NaOH, $NH_4OH$, $H_2O$; c) concentrated HCl, acetone.*

After the Schiff base formation, imine hydrolysis and subsequent precipitation using concentrated hydrochloric acid in acetone, the desired amino acid amide **19** and **20** were obtained as the corresponding HCl-salts. As with the previous sequence, the final precipitation step allows scaling up of the whole sequence without the necessity of performing any column chromatography.

## *2.5 Enzymatic resolution*

The enzymatic resolutions of the obtained amino acid amides were carried out following procedures that were previously developed by our group in collaboration with DSM.[29] This involved the use of whole cells of *Pseudomonas putida* ATCC 12633 or whole cells of a genetically modified *Escherichia coli* strain (GMO), both of which contain an aminopeptidase that displays an

excellent enantioselectivity in combination with a high tolerance towards the size and functional groups of the side chain.




| | yield ((*S*) ee) (%) | yield ((*R*) ee) (%) |
|---|---|---|
| 2-amino-pent-4-ynoic acid | 39 (>96) (*S*)-**4** | 38 (>96) *(R)*-**4** |
| 2-amino-hex-4-ynoic acid | 40 (>96) (*S*)-**13** | 42 (>99) *(R)*-**13** |
| 2-amino-hept-5-ynoic acid | 40 (>96) (*S*)-**14** | 41 (>96) *(R)*-**14** |
| 2-amino-oct-6-ynoic acid | 44 (>96) (*S*)-**15** | 43 (>99) *(R)*-**15** |

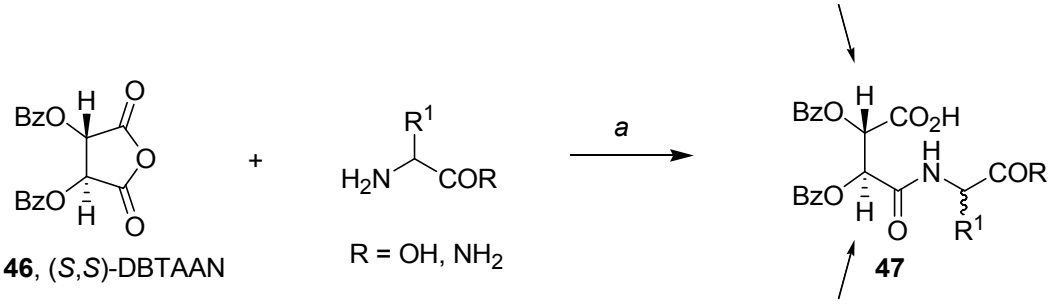
**Scheme 11**. *Reagents and conditions: a) Whole cells from Pseudomonas putida ATCC 12633 or from an Escherischia coli GMO, 80 mM aqueous solution of $MnSO_4$, pH = 9.2, 37 °C; b) Whole cells from Rhodococcus erythropolis NCIMB 11540, aqueous buffered ($KH_2PO_4$) solution of pH = 8.0.*

The enzymatic resolutions were conducted at 40 °C and pH = 9.2 in the absence of sodium and chloride ions. These ions are known to significantly lower the aminopeptidase activity. Therefore prior to the resolution these salts were removed using ion exchange chromatography. A 10% solution (by weight) of the amide in water was treated with whole cells from the *E. coli* GMO in a substrate to cell mass ratio of 500 : 1. During the reaction, small reaction samples were taken and analyzed via chiral HPLC analysis in order to monitor the conversion and the enantiomeric excess.

After the reaction was completed, the mixture of (*R*)-amide and (*S*)-acid was separated by the earlier described procedure of Schiff base formation and subsequent extraction. The optically pure (*S*)-amino acid was obtained from the

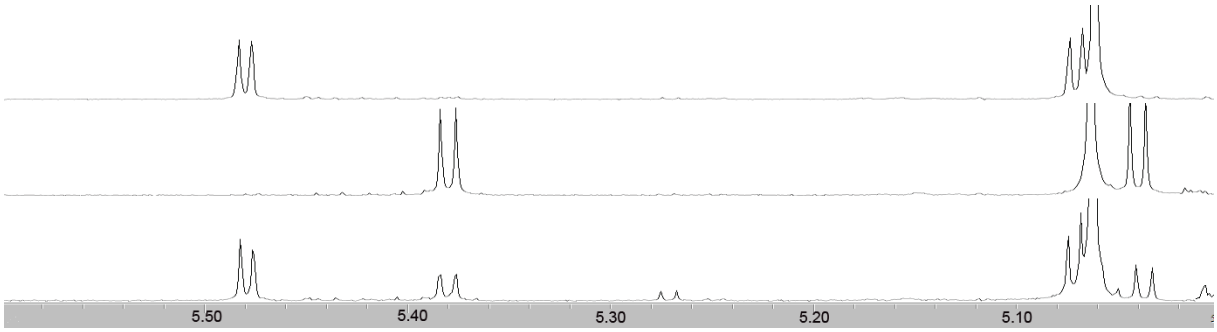
aqueous layer and was further purified using ion exchange chromatography. The Schiff base of the (*R*)-amide was obtained via evaporation of the organic layer and subsequent hydrolysis and precipitation using equimolar concentrated HCl in acetone. Transformation of the (*R*)-amide into the corresponding (*R*)-acid was achieved using *Rhodoccocus erythropolis* NCIMB 11540 whole cells, containing a non-specific amidase. Thus, subjection of the (*R*)-amino acid amides (pH = 8.0, T = 37 °C) to these whole cells led to full conversion into the corresponding amino acids as monitored by TLC (eluent: $CHCl_3$ : MeOH : $NH_4OH$ 6 : 3 : 1). Filtration over Celite to remove the cell remainders, followed by further purification using ion exchange chromatography delivered the optically pure (*R*)-amino acids **4**, **13**-**15**. The yield and enantiomeric purity of the different products are summarized in Scheme 11. Generally, excellent selectivities were observed in combination with good overall yields for these processes.

The ee's of the amino acids obtained from the enzymatic resolution were determined using an *in situ* reaction with the tartaric acid-derived enantiopure anhydride (*S*,*S*) dibenzoyltartaric acid anhydride (DBTAAN (**46**)) and concomitant $^1$H−NMR analysis of the resulting diastereoisomers.[30]




**Scheme 12**. *Reagents and conditions: a) $Na_2B_4O_7$, $CD_3CN$ : $D_2O$ (1 : 1).*

A racemic amino acid (*viz. rac*-**13**) gives rise to two diastereoisomers, whereas an optically pure amino acid will solely give one diastereoisomer. Upon integration of the α-protons, the enantiomeric excess can be determined.




**Scheme 13**. *Example of chemical shifts: entry 1: pure (S)*-**13**, *entry 2: pure (R)*-**13**, *entry 3: (~2 : 1) mixture.*

Scheme 13 shows an example. The $^{1}$H−NMR spectra show the α-anhydride protons of the resulting diastereoisomers. Both the anhydride and the amino acid α-proton shift to 5.06 ppm, while the (*S*)-**13** amide α-proton is found at 5.48 ppm whereas *(R)*-**13** is found at 5.38 ppm. A mixture of 2 : 1 of both enantiomers as pseudoracemic reference shows the ratio in the NMR-values of both amino acids. This method can be in principle generally applied for amino acids, thus allowing $^{1}$H−NMR as a means for measuring optical purities.

## *2.6 Conclusions*

In this chapter the overall synthesis of optically pure unsaturated amino acids has been outlined. Either alkylation of the O'Donnell ketimine (for **13**) or a modified Strecker reaction led to the required amino acid amides for the enzymatic resolution. During these syntheses several improvements were implemented as compared to previous work. The resulting amino acids have been used throughout the following chapters.

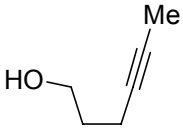
## *2.7 Acknowledgements*

Roy Storcken, Jan Dommerholt and Denis IJzendoorn are kindly acknowledged for performing the resolution experiments performed at DSM (Geleen, The Netherlands) under the guidance of Theo Sonke.

## *2.8 Experimental section[31]*

$^{1}$H−NMR (300 MHz) and $^{13}$C−NMR (75 MHz) spectra were recorded on a Bruker DMX-300 spectrometer in $CDCl_3$ using tetramethylsilane as internal standard (unless stated otherwise). $^{1}$H−NMR (400 MHz) and 2D−NMR experiments (NOESY, COSY) were performed on a Varian Unity Inova-400 spectrometer in

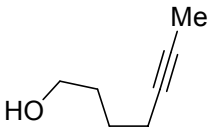
$CDCl_3$. All NMR spectra were recorded at 298 K, unless stated otherwise.[32] Chemical shifts are reported in parts per million (ppm). Mass spectra were recorded on a VG7070E double-focusing mass spectrometer using EI and CI modes. IR spectra were recorded on a Anadis Thermo Mattson IR300 spectrometer. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plates. Spots were visualized with UV, exposure to iodine vapor or by either dipping the TLC plate into a 6.2% aqueous sulfuric acid solution containing ammonium molybdate (42 g $L^{-1}$) and ceric ammonium sulfate (3.6 g $L^{-1}$) or potassium permanganate solution (made by 1.5 g $KMnO_4$, 10 g $K_2CO_3$ and 2 mL 5% NaOH in 150 mL $H_2O$) followed by charring. Column chromatography was carried out with Acros silica gel (0.035-0.070mm).[33] Ion exchange chromatography was performed using Dowex 50W×4 $H^+$-form (20-50 mesh, Fluka). Melting points were determined on a Buchi B-545 melting point apparatus and are uncorrected. When necessary, reactions were performed under standard Schlenk conditions under an argon atmosphere. Most commercially available solvents and reagents were used as received. EtOAc and heptane were distilled prior to use. THF and $Et_2O$ were dried by distillation from sodium and benzophenone. Raney® 2800 nickel was purchased from Aldrich and was extensively washed with methanol prior to use.



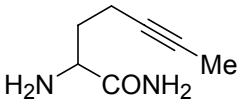

**4-Hexynol (38).** To a solution of $NaNH_2$ (90% dispersion in mineral oil, 108 g, 2.48 mol) in liquid $NH_3$ (~1 L) was added dropwise a solution of 2-chloromethyltetrahydrofuran (**34**, 100 g, 0.829 mol) in $Et_2O$ (50 mL). The resulting solution was stirred at –33 °C for 6 h. The reaction was quenched by addition of saturated aqueous ammonium chloride (500 mL) and the ammonia was allowed to evaporate overnight. The reaction mixture was extracted using $Et_2O$ (3 × 500 mL), the organic layers were combined and washed (brine), dried ($Na_2SO_4$) and evaporated. The crude oil **36** (70.35 g, 0.83 mol) was used without further purification in the subsequent methylation step.

To a solution of $Fe(NO_3)_3 \cdot 9H_2O$ (200 mg, 0.63 mmol) in liquid $NH_3$ (1 L) small turnings (ca. 1 g) of freshly cut lithium (14.0 g, 2.00 mol) were added whenever the color changed from blue to grey. Then, 4-pentynol (70.4 g, 0.83 mol) was added dropwise using a syringe over a period of 30 min. After stirring for 1 h, MeI (51.8 mL, 0.820 mol) was slowly added. The mixture was quenched using saturated aqueous ammonium chloride (400 mL) and the ammonia was allowed to evaporate overnight. The resulting mixture was extracted using $Et_2O$ (3 × 600 mL), dried ($MgSO_4$) and evaporated. Further purification using vacuum

distillation (300 mbar, 50 °C) yielded **38** as a colorless oil (56.7 g, 0.58 mol, 70% over 2 steps). $^1$H−NMR (400 MHz, $CDCl_3$) δ 3.73 (t, *J* = 6.1 Hz, 2H, C*H*$_2$OH), 2.25–2.20 (m, 2H, C≡CC*H*$_2$), 1.85 (br s, 1H, OH), 1.75 (t, *J* = 2.6 Hz, 3H, C≡CMe), 1.74–1.67 (m, 2H, HOCH$_2$C*H*$_2$).




**5-Heptynol (39).** Using the previously described method, 2-chloromethyltetrahydropyran (**35**, 75.0 g, 0.557 mol) was ring opened and methylated to yield after vacuum distillation (17 mbar, 105 °C) **39** as an oil (50.6 g, 0.451 mol, 81% over 2 steps). $^1$H−NMR (400 MHz, $CDCl_3$) δ 3.68–3.65 (m, 2H, C*H*$_2$OH), 2.19–2.14 (m, 2H, C≡CC*H*$_2$), 1.77 (t, *J* = 2.6 Hz, 3H, C≡CMe), 1.68–1.60 (m, 2H, HOCH$_2$C*H*$_2$), 1.68–1.60 (m, 2H, C*H*$_2$), 1.38 (br s, 1H, OH).

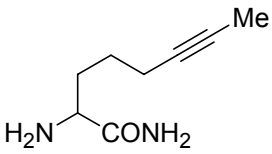



**2-Amino-5 heptynoic acid amide (19).** To a solution of oxalyl chloride (90 mL, 0.965 mol) in $CH_2Cl_2$ (1.25 L) at –78 °C was added slowly freshly distilled DMSO (87 mL, 1.24 mol). After stirring for 10 min, a solution of 4-hexynol (**38**, 56.6 g, 0.577 mol) in $CH_2Cl_2$ (50 mL) was added slowly and the reaction was stirred for an additional hour at –78 °C. Then freshly distilled $Et_3N$ (282 mL, 2.01 mol) was added and the reaction was allowed to warm to room temperature while vigorously stirred. The reaction was quenched by adding aqueous saturated ammonium chloride (400 mL) and the mixture was extracted using $Et_2O$ (3 × 400 mL). The combined organic layers were washed (brine), dried over $Na_2SO_4$ and gently evaporated since the aldehyde was rather volatile. The crude 4-hexynal (**21**) was obtained as a yellowish oil and directly used in the Strecker reaction.

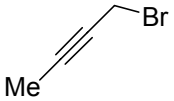
To a solution of NaCN (28.3 g, 0.577 mol) in $NH_4OH$ (25 M, 500 mL) and $H_2O$ (200 mL) was added $NH_4Cl$ (30.28 g, 0.577 mol) and the resulting solution was stirred for 30 min after which the crude aldehyde in MeOH (50 mL) was added. The reaction mixture was stirred for 4 h at 40 °C. It was extracted with $Et_2O$ (3 × 500 mL) and the combined organic layers were concentrated *in vacuo* to give the crude aminonitrile as a yellow oil. Caution is required since this can undergo a retro reaction releasing hydrogen cyanide.

The crude oil was redissolved in $NH_4OH$ (25 M, 500 mL) diluted with $H_2O$ (200 mL) and treated with 10 M NaOH (57.7 mL, 0.577 mol) and benzaldehyde (56.0 mL, 0.577 mol). The resulting mixture was stirred for 4 h at ambient temperature. The reaction mixture was extracted using $CH_2Cl_2$ (3 × 400 mL), the combined organic layers were washed (brine, 200 mL) and concentrated. The

crude Schiff base thus obtained was dissolved in acetone (350 mL) and treated with concentrated hydrochloric acid (37%, 50.8 mL, 0.577 mol). The resulting precipitation was collected by filtration and washed using acetone (100 mL), yielding the desired amino acid amide **19** (35.4 g, 0.201 mol, 34%) as a white-powdered HCl-salt. $^1$H–NMR (400 MHz, $D_2O$) δ 4.16 (t, *J* = 6.8 Hz, 1H, CαH), 2.39–2.35 (m, 2H, C≡C$CH_2$), 2.14–2.03 (m, 2H, CαC$H_2$), 1.78 (t, *J* = 2.5 Hz, 3H, C≡CMe).

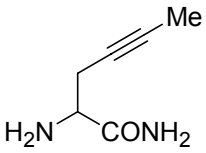



**2-Amino-6-octynoic acid amide (20).** 5-Heptynol (**28**, 20.0 g, 0.178 mol) underwent the previously described transformations to yield eventually the desired amino acid amide **20** (25.5 g, 0.134 mol, 75%) as a white-powdered-HCl salt. $^1$H–NMR (400 MHz, $D_2O$) δ 4.07 (t, *J* = 6.5 Hz, 1H, CαH), 2.26–2.23 (m, 2H, C≡C$CH_2$), 2.03–1.98 (m, 2H, $CH_2$), 1.77 (t, *J* = 2.5 Hz, C≡CMe), 1.62–1.57 (m, 2H, $CH_2$).



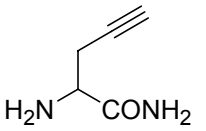

**1-Bromo-2-butyne (27).** To a solution of $Fe(NO_3)_3 \cdot 9H_2O$ (100 mg, 0.24 mmol) in liquid $NH_3$ (1 L) small turnings (ca. 1 g each) of freshly cut Li (20.0 g, 2.86 mol) were added when the color changed from blue to grey. Propargyl alcohol (70.0 g, 1.20 mol) was added dropwise over a period of 30 min. After stirring for 1 h, MeI (75.0 mL, 1.20 mol) was slowly added. The mixture was quenched after 2 h using saturated aqueous ammonium chloride (400 mL) and the ammonia was allowed to evaporate overnight. The resulting mixture was extracted using $Et_2O$ (3 × 500 mL), dried ($MgSO_4$) and evaporated to give **26** (64.5 g, 0.89 mol) as a yellow oil. $^1$H–NMR (400 MHz, $CDCl_3$) δ 4.24–4.21 (m, 2H, $CH_2OH$), 1.86 (t, *J* = 2.4 Hz, 3H, Me).

The crude oil **26** (64.5 g, 0.89 mol) was dissolved in $Et_2O$ (240 mL) and pyridine (12 mL) was added. After cooling to –78 °C, $PBr_3$ (32.4 mL, 340 mmol) was added dropwise and the mixture was stirred vigorously for 2 h. The reaction mixture was slowly warmed to ambient temperature and subsequently refluxed for 30 min. After quenching with brine (100 mL), the mixture was extracted with $Et_2O$ (3 × 150 mL) and concentrated *in vacuo*. Purification via distillation (bp: 130 °C) yielded **27** as a colorless oil (79.5 g, 0.60 mol, 50%). $^1$H–NMR (300 MHz, $CDCl_3$) δ 3.91 (q, *J* = 2.5 Hz, 2H, $CH_2Br$), 1.89 (t, *J* = 2.6 Hz, 3H, Me); $^{13}$C–NMR (50 MHz, $CDCl_3$) δ 83.5, 74.3, 15.6, 3.7.

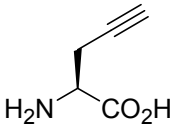


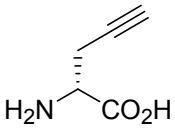

**2-Amino-4-hexynoic acid amide (18).** To a solution of glycine diphenylketimine methyl ester **16** (80.1 g, 317 mmol) and LiI (5.0 g, 31.7 mmol) in THF (1 L) was added NaH (12.7 g of a 60% dispersion in mineral oil, 317 mmol). After heating to reflux, freshly distilled 1-bromo-2-butyne (**27**, 30.5 ml, 360 mmol) in THF (50 mL) was added dropwise over a period of 45 min. The reaction was refluxed for 2 h and after cooling to room temperature quenched using aqueous saturated ammonium chloride (600 mL). The reaction mixture was extracted using $Et_2O$ (3 × 700 mL) and the organic phase was washed (brine, 500 mL), dried ($Na_2SO_4$) and evaporated, yielding **29** as a dark oil (111 g, 311 mmol) in 98% yield.

The crude oil (110 g, 360 mmol) was dissolved in $Et_2O$ (1 L) and aqueous hydrochloric acid was added (1 M, 340 mL) at 0 °C. The resulting mixture was vigorously stirred overnight at room temperature. The resulting mixture was extracted using water and the combined aqueous layers were evaporated, yielding a yellowish solid. The solid thus obtained was dissolved in ammonia (25%, 800 mL) and after stirring for 2 h the mixture was evaporated. The solid was redissolved in water (500 mL) and after the addition of benzaldehyde (33.5 g, 317 mmol) stirring was continued overnight. The aqueous layer was extracted with $CH_2CL_2$ (3 × 500 mL) and the combined organic layers were washed (brine), dried ($MgSO_4$) and evaporated. Then the oil was dissolved in acetone and aqueous hydrochloric acid (37%, 25 mL) was added dropwise, causing the desired amide to precipitate. After filtration the residue was washed using acetone, thus yielding **18** (37.0 g, 227 mmol, 70.1%) as a white solid. $^1$H−NMR ($D_2O$, 300 MHz) δ 3.55 (t, *J* = 5.8 Hz, CαH), 2.55–2.53 (m, 2H, C≡C$CH_2$), 1.79 (t, *J* = 2.5 Hz, C≡CMe).




**2-Amino-4-pentynoic acid amide (17).** Using the previously described method, glycine diphenylketimine methyl ester (**16**, 20.0 g, 79.0 mmol) was alkylated with propargyl bromide (12.8 g, 103 mmol) yielding **17** (23.0 g, 78.0 mmol) as a yellowish oil. Further treatment as described previously yielded after ion exchange chromatography (Dowex 50W×4 $H^+$-form, 20−50 mesh, Fluka) a yellowish powder (5.18 g, 46.0 mmol, 78%). $^1$H−NMR ($D_2O$, 400 MHz) δ 4.37 (t, *J* = 5.8 Hz, 1H, CαH), 3.07–3.05 (m, 2H, C≡C$CH_2$), 2.75 (t, *J* = 2.1 Hz, 1H, C≡CH).

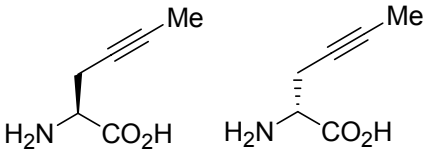


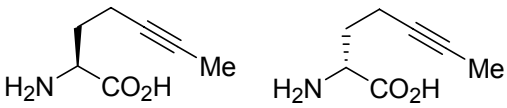

**General procedure for the enzymatic resolution, exemplified for 2-amino-4-pentynoic acid (4).** A solution of the racemic

amide **17** (12.0 g, 105 mmol) in distilled $H_2O$ (80 mL) was brought to pH = 9.2 with 1 N aqueous KOH, followed by addition of an 80 mM solution of $MnSO_4$ until a 1 mM concentration was reached. $H_2O$ was added until a volume of 120 mL was reached. Then whole *E. coli DH5α/ pTrpLAP* cells (48 mg) in a HEPES buffer (1 mL) were added. The reaction mixture was shaken at 40 °C for 24 h. The reaction mixture was brought to pH = 6 by carefully adding $H_2SO_4$. The enzyme was filtered off the solution, and by adding NaOH the pH was brought at 8–9; then benzaldehyde (7.5 mL, ~0.7 equiv) was added and the resulting mixture was stirred for 4 h. The mixture was extracted using $CHCl_3$ and the aqueous layer was concentrated and purified on ion exchange chromatography (Dowex 50). Upon lyophilization, (*S*)-**4** was obtained as a white powder (4.7 g, 42 mmol, 39%).

The organic layer was concentrated and redissolved in acetone (300 mL) after which aqueous concentrated HCl was added (~6 mL, 37%), causing a precipitation. After filtration the HCl-salt of (*R*)-amino acid amide **17** (6.0 g, 41.0 mmol, 38%) was obtained. The (*R*)-amino acid amide was dissolved in buffer (concentration 5%, pH = 8 (500 mL 0.1 M $NaH_2PO_4$ + 467 mL 0.1 M NaOH). Enzyme extracts from *Rhodoccocus erythropolis* (NCIMB 11540, 0.2 equiv in weight) were added and the reaction mixture was stirred at 37 °C for 3h. The reaction mixture was filtered over a short path of Celite, acidified to pH = 5, concentrated and further purified by ion exchange chromatography (Dowex 50). Concentration using freeze-drying yielded (*R*)-**4** as a white fluffy solid (4.6 g, 40 mmol, 38%).$^1$H–NMR ($D_2O$, 400 MHz) δ 4.37 (t, *J* = 5.75 Hz, 1H, CαH), 3.06–3.05 (m, 2H, C≡C$CH_2$), 2.75 (t, *J* = 2.4 Hz, 1H, C≡CH); $^{13}$C–NMR (100 MHz, $D_2O$) δ 175.5, 77.1, 76.3, 55.7, 23.3.

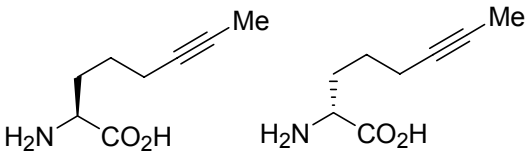



**2-Aminohex-4-ynoic acid (13).** Using the previously described method for enzymatic resolution, **18** (20.2 g, 0.124 mol) was converted in (*S*)-**13** (6.22 g, 49 mmol, 40%) and (*R*)-**13** (6.55 g, 52 mmol, 42%), both as a fluffy white solid. $^1$H–NMR ($D_2O$, 400 MHz) δ 3.85–3.83 (m, 1H, CαH), 2.77–2.75 (m, 2H, C≡CCH2), 1.77 (t, J = 2.4 Hz), 3H, C≡CMe); $^{13}$C–NMR (100 MHz, $D_2O$) δ 175.9, 84.4, 74.7, 56.1, 23.6, 5.2.




**2-Aminohept-5-ynoic acid (14)**. Using the previously described method for enzymatic resolution, **19** (14.1 g, 80 mmol) was converted in (*S*)-**14** (4.49 g, 32 mmol, 40%) and (*R*)-**14** (4.73 g, 33 mmol, 41%),

both as a fluffy white solid. $^1$H−NMR ($D_2O$, 400 MHz) δ 3.84 (dd, J = 5.2 Hz, 7.4 Hz, 1H, CαH), 2.40−2.21 (m, 2H, C≡CCH2), 2.19−2.07 (m, 1H, $C\alpha CH_2$), 2.05−1.94 (m, 1H, $C\alpha CH_2$), 1.77 (t, *J* = 2.41 Hz, 3H, C≡CMe); $^{13}$C−NMR (100 MHz, $D_2O$) δ 176.0, 82.0, 79.5, 55.1, 32.6, 17.0, 5.2.




**2-Aminooct-6-ynoic acid (15).** Using the previously described method for enzymatic resolution, **20** (22.0 g, 0.115 mol) was converted in (*S*)-**15** (7.87 g, 51 mmol, 44%) and (*R*)-**15** (7.69 g, 50 mmol, 43%), both as a fluffy white solid. $^1$H−NMR ($D_2O$, 400 MHz) δ 3.76 (t, *J* = 6.1 Hz, 1H, CαH), 2.25−2.21 (m, 2H, $C\equiv CCH_2$), 2.11−1.88 (m, 2H, $CH_2$), 1.77 (t, *J* = 2.5 Hz, 3H, C≡CMe), 1.68−1.48 (m, 2H, $CH_2$); $^{13}$C−NMR (100 MHz, $D_2O$) δ 174.9, 81.7, 80.8, 55.5, 32.7, 26.3, 20.3, 5.1.

*General procedure for (S,S)-DBTAAN mediated ee determinations.*
In a NMR−tube, the amino acid (4 mg) was mixed with 0.4 M $Na_2B_4O_7$ (0.5 mL in $D_2O$) and (*S*,*S*)-DBTAAN (0.5 mL, 50 mM in $CD_3CN$). After 3 h, $^1$H−NMR was recorded using $CD_3CN$ as lock signal. For the ee-determination, the integral values of the anhydride α-proton were compared to a reference racemic mixture.

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# 3 CONSTRAINED CYSTINE MIMICS

## *3.1 Introduction*

Introduction of covalent linkage in peptide structures in order to stabilize the tertiary structure is a feature occurring in Nature. This mostly proceeds through the formation of sulfur bridges resulting in cystine (**1**) cross-links, which, however, are susceptible to natural degradation pathways. Hence, obvious artificial constraints in peptides might be based on the corresponding carbon analogues **2**, e.g. diaminosuberic acid-derived cross-links (Figure 1).

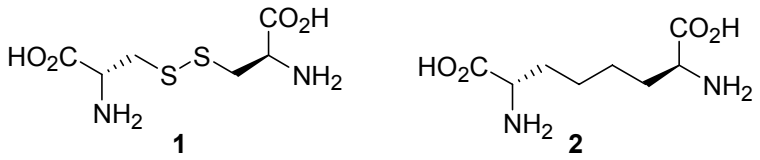



**Figure 1**. *Cystine (1) and diaminosuberic acid (2).*

Nevertheless, to create a good mimic, the properties of the carbon bridge should not deviate too much from its sulfur counterpart. In comparing the sulfur to the carbon bridge, there is a difference in flexibility, but also in the electronic properties. The group of Tirrell investigated the *in vivo* substitution of methionine with a series of (un)saturated amino acids with side chains of various lengths.[1] It was shown that the acetylene moiety served particularly well as a mimic of the methyl thioether moiety of methionine in bacterial protein synthesis. Calculated equipotential surfaces on methionine showed that the electron density associated with the triple bond is positioned similar to that of the thioether of methionine, despite the difference in geometry of the side chain.
Taking this into account, we performed similar orbital modeling on cystine and its alkyl, olefin and alkyne counterparts (Figure 2).[2] The electron density is concentrated at the sulfur bridge due to the sulfur d-orbitals. This electron density is absent in the saturated carbon isostere **2** (the observed density is due to proximity effects of the functional groups) and rather minute in its olefin counterpart **3**. The alkyne isostere **4**, however, shows a clear band around the unsaturation, caused by the sp-hybridization of the carbons.

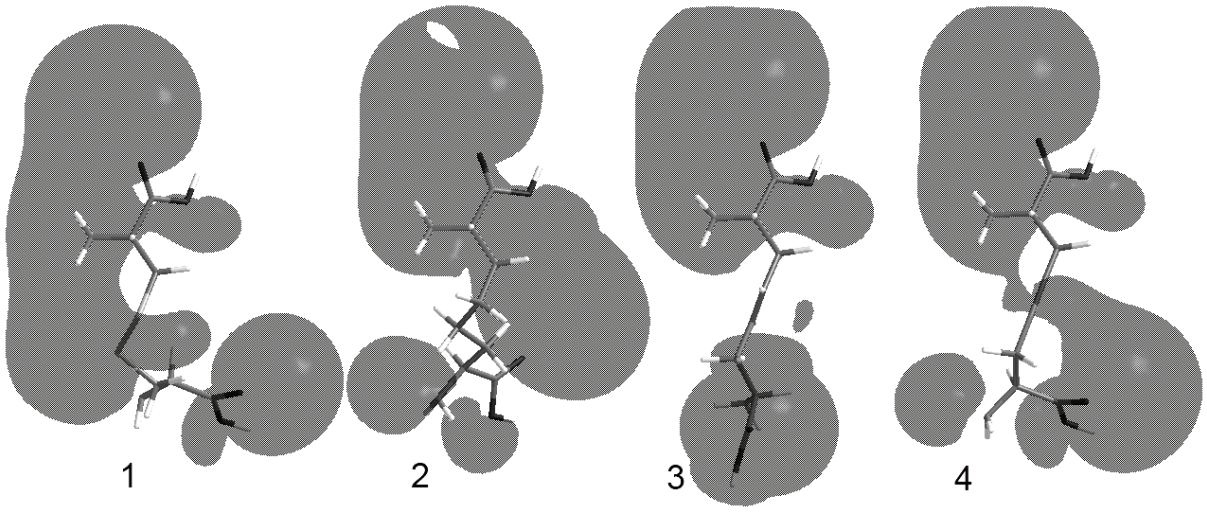
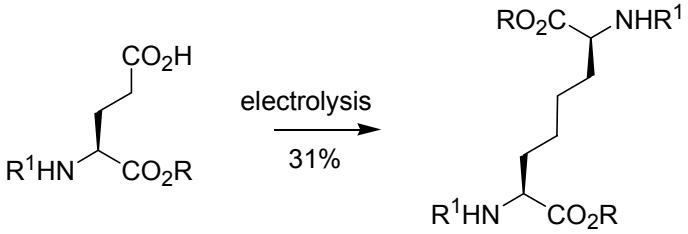


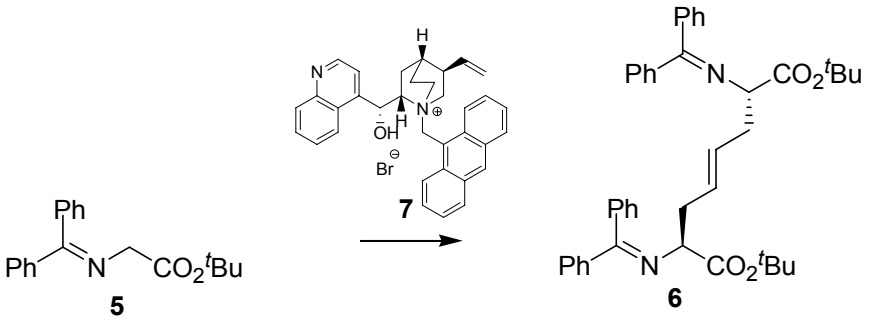

**Figure 2.** *Electron potentialsurfaces: cystine (**1**), the alkyl (**2**), alkene (**3**) and alkyne (**4**) isosteres.*[3]

Currently, several synthetic routes to diaminosuberic acid derivatives are available, which are briefly surveyed in this section. Most of these syntheses consist of two (protected) glutamates, which are connected using the Kolbedimerization (Scheme 1).[4] This reaction proceeds via electrolysis, generating an unstable radical on the anode, that after loss of $CO_2$ results in dimerization. A significant drawback of this approach is that orthogonal protection is difficult due to the formation of statistical mixtures of products. Apart from this, the dimerization proceeds generally in low yields.



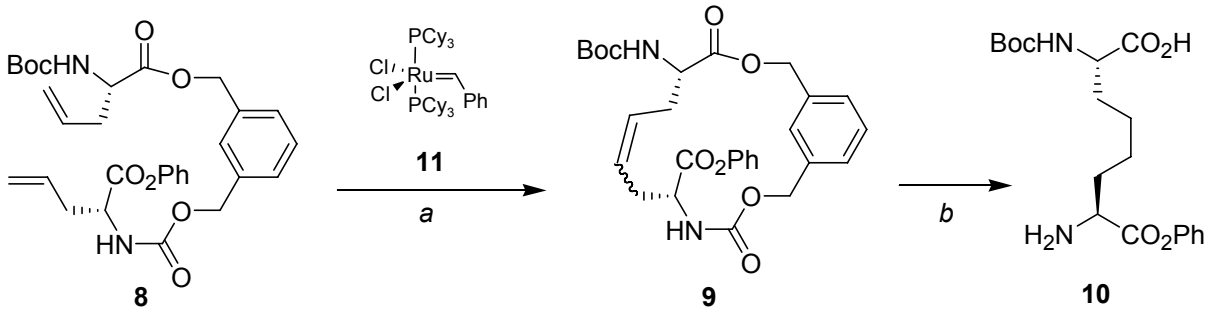

**Scheme 1**. *Kolbe electrolysis.*

A synthesis of the Peterson group involves use of the chiral catalyst **7** to generate two new stereocenters in a double asymmetric alkylation of the glycine-derived ketimine **5** (Scheme 2).[5] An interesting drawback is the directing effect of the first stereocenter in the second alkylation, causing that the diastereoselectivity is significantly lower than the enantioselectivity of the first step.




**Scheme 2**. *Reagents and conditions: Catalyst* ***7*** *(10 mol %) 50% aqueous KOH/toluene, (E)-1,4-dibromo-2-butene (de 82%, ee >95%).*

These two examples give rise to diaminosuberic acid, but the drawback of orthogonal protection remains. There is no discrimination possible between the identical functional groups. To solve this problem each stereocenter has to be generated independently from differently protected glycine moieties or the two amino acid moieties must be linked via the side chains using appropriate orthogonal protective groups. An example by Williams *et al.* of the latter approach is shown in Scheme 3,[6] where two allylglycine moieties are connected to a tether in an asymmetric fashion. Ring-closing metathesis, followed by deprotection leads to orthogonally protected diaminosuberic acid derivative **10**.

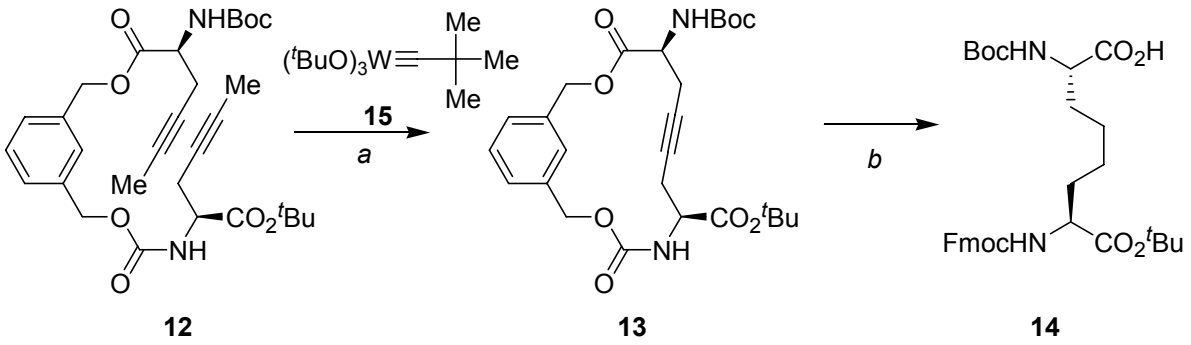



**Scheme 3**. *Reagents and conditions: a) catalyst* ***11****, $CH_2Cl_2$ (82%); b) i: $H_2$, Pd/C; ii: pTSA, $H_2O$.*

Apart from the fact that this is a lengthy route, the metathesis reaction gives rise to a mixture of (*E*)- and (*Z*)-isomers (*viz.* **9**). To solve this, the resulting double bound is often reduced with concomitant removal of the benzyl protective groups. However, besides the fully saturated structure – which is more flexible than cystine and lacks its electronic properties – one would also like to have access to the unsaturated suberic acid derivatives in geometrically pure form.

Acetylene-based diaminosuberic acid derivatives such as alkyne isostere **4** (Figure 2) are even more interesting mimics of cystine, especially considering the electron potential surfaces. The latter class of molecules can be formed via ring-closing alkyne metathesis, in an approach that resembles the one of Williams

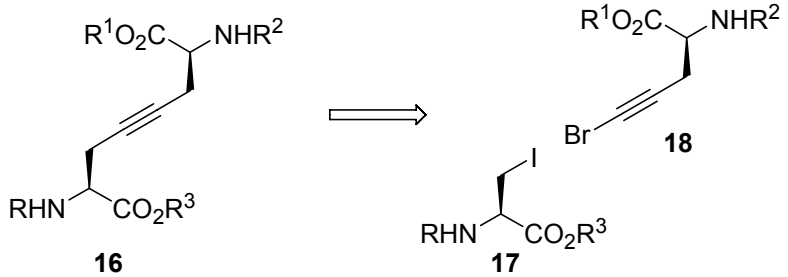
(Scheme 4). Proof for this concept was generated in our own group in a collaborative effort,[7] leading to well-defined cystine mimics **13** and **14** with comparable rigidity. Although this sequence was applied to form olefin- and acetylene-containing diaminosuberic acid derivatives, we have not been fully able to prepare orthogonally protected unsaturated cystine mimics.




**Scheme 4**. *Reagents and conditions: a)* ***15****, PhCl, 80 °C (66%); b)* $H_2$*, Pd/C then FmocOSu (76%).*

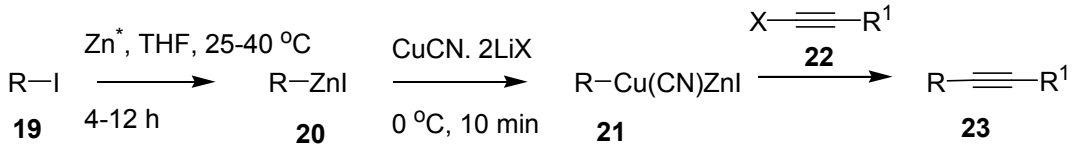
Thus, in order to create access to the desired unsaturated orthogonally protected cystine mimics, a direct C-C bond forming reaction between two optically active amino acid fragments would be the most straightforward method. Considering potential pitfalls such as orthogonal protection, racemization of the stereocenters and vulnerability of the acetylene bond, the available synthetic tools that might be applied are rather limited.

General methods to make sp-$sp^3$ C-C bonds involve deprotonation of the acetylene moiety using a strong base (typically BuLi or EtMgBr), followed by nucleophilic attack on a suitable alkylating reagent. Obviously, this sequence cannot easily be applied to amino acid synthesis due to the risk of racemization of the amino acid stereocenters. Therefore, alternative activation of the functional groups involved is required. Interestingly, in searching the literature for suitable methodology, only a limited number of methods was identified that could be applied to our situation. We envisioned that the coupling of a haloacetylene with an iodide-derived Zn-Cu reagent would be a sufficiently mild method to reach our goal (Scheme 5).



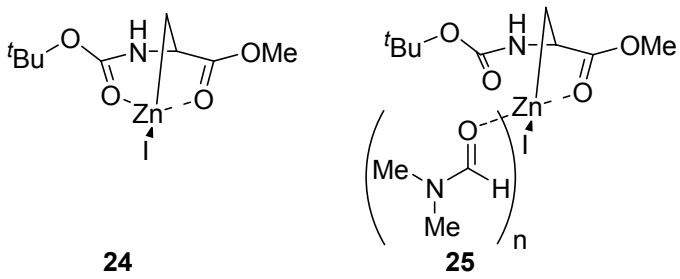

**Scheme 5**. *Retrosynthesic approach to unsaturated cystine mimics.*

Early reports by Knochel indicate that zinc/copper reagents do not react with ketones,[8] esters and nitriles and react under generally mild reaction conditions with suitable electrophiles. As shown in Scheme 6, a primary or secondary iodide (**19**) can be transformed into the stable organozinc compound **20**, which after transmetalation to the copper complex **21**, can react with a haloacetylene (**22**) to form the new acetylene **23**.




**Scheme 6**. *Formation zinc/copper reagents.*

Extensive research on zinc activation of iodonated amino acids has been performed by the group of Jackson.[9]

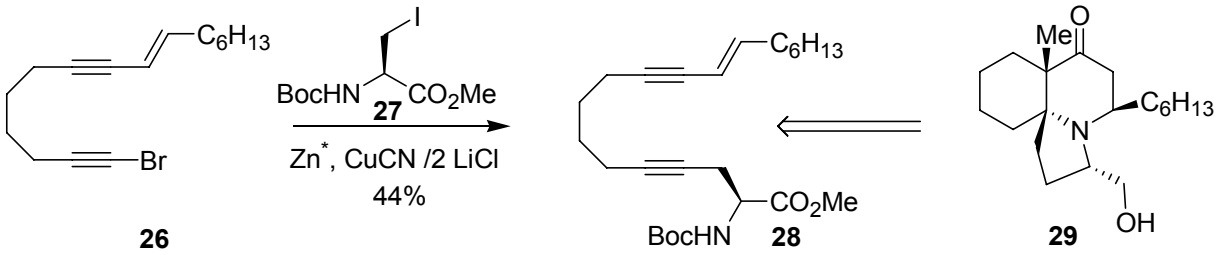



**24** **25**

**Scheme 7**. *Stabilization of the zinc intermediate in DMF.*

Coordination of the carbamate carbonyl (*viz.* **24**) in THF is expected to give elimination to form an acrylate,[10] but since a bidentate complex with the ester carbonyl is formed, the elimination is prevented. This was proven by comparison with the corresponding ß- and γ-amino acid-derived organozinc/copper species, where the stabilizing effects were less pronounced and thus more elimination was observed. This was further underlined by the observation that replacement of the ester by an amide also gave a decrease of the stabilizing effect. Titration experiments with DMF showed a progressive weakening of the coordination of zinc with the carbamate and strengthening of the coordination to the ester group (*viz.* **25**).[11] Although both THF and DMF are suitable solvents for organozinc reagents, this result suggests that in this case DMF is the solvent of choice.

In the course of our work,[12] a successful application was published by the Trost group,[13] in which the (*S*)-serine-derived iodide **27** was coupled to the acetylenic precursor **26** to undergo a hydrative cyclization using ruthenium catalysis leading to the carbon skeleton of cylindricin C (**29**).

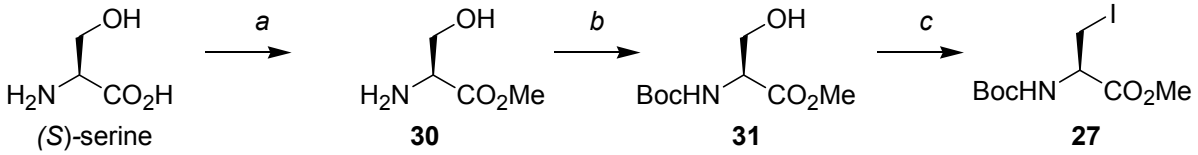



**Scheme 8**. *Zn/Cu-mediated C-C bond formation in the synthesis of cylindricin C (**29**).*

This chapter details with our investigation on the potential use of amino acid-derived organozinc reagents in the formation of sp-sp$^3$ C-C bonds with the aim to prepare new acetylene-containing amino acids (Scheme 5). These unsaturated amino acids should then serve to prepare orthogonally protected diaminosuberic acid derivatives.

## *3.2 Starting materials*

(*S*)-Serine was protected in two steps to give **31**, which is a suitable precursor to form the iodinated counterpart **27**.[14] Initial attempts to form the iodide using triphenylmethylphosphonium iodide failed, but treatment with triphenylphosphine, iodine and imidazole, as shown in Scheme 9, eventually yielded the desired compound **27** in reasonable yield.

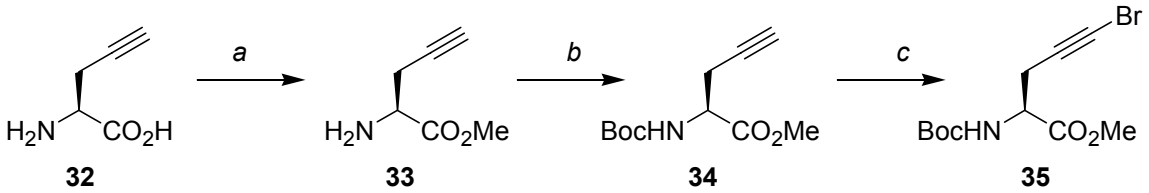



**Scheme 9**. *Reagents and conditions: a) $SOCl_2$, MeOH, reflux; b) $Boc_2O$, $Et_3N$, THF (99%, 2 steps); c) $PPh_3$, imidazole, $I_2$, $CH_2Cl_2$ (72%).*

An alternative approach relied on tosylation of **31** and subsequent nucleophilic attack of NaI, but this gave compound **27** in a much lower yield due to elimination.

As a coupling partner, racemic 2-amino-4-pentynoic acid (**32**) was chosen to probe our approach (Scheme 10). Standard protecting group introduction delivered compound **34** which was subjected to the halogenation conditions. While a combination of iodine and morpholine gave no reaction, treatment with a catalytic amount of silver nitrate and *N*-bromosuccinimide (NBS) successfully provided the bromoacetylene **35** in 72% yield. Eventually this latter method

proved a reliable and convenient method for introducing the halide substituents on the acetylene under mild conditions.

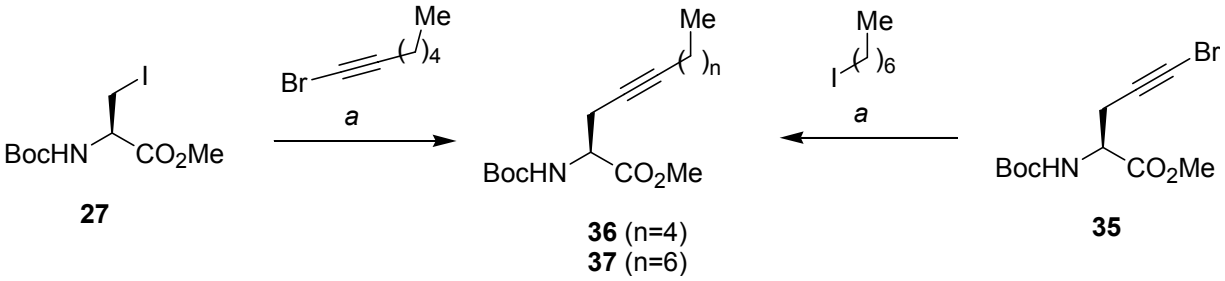



**Scheme 10**. *Reagents and conditions: a) $SOCl_2$, MeOH, reflux; b): $Boc_2O$, $Et_3N$, THF (88%, 2 steps); c) $AgNO_3$, NBS, acetone (72%).*

This reaction also proceeded well with *N*-iodo instead of *N*-bromosuccinimide, but the resulting iodoacetylene appeared to be unstable and fully decomposed in several hours. The bromide on the other hand was stable and did not show any decomposition upon storage at 0 °C for weeks.

## *3.3 Benchmark studies*

In preparing the acetylenic amino acids (*viz*. **36** and **37**), one could on the one hand start from serine-derived iodide **27** and 1-bromo-1-heptyne, and on the other hand from 1-iodopentane and **35** (Scheme 11). Both will lead to the same compound, but start from different amino acids. Since 1-iodoheptane cannot undergo ß-elimination and is commercially available, initial experiments were performed on the latter pathway.



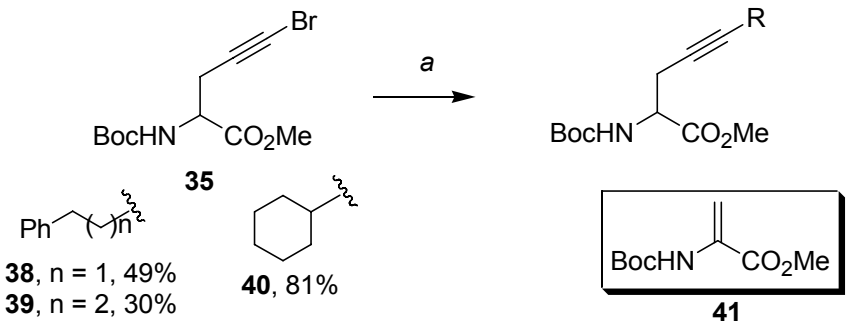

**Scheme 11**. *Reagents and conditions: a) Zn*, CuCN/2·LiCl, DMF.*

In all cases, so-called Riecke zinc (indicated with Zn*: zinc (99.998%) treated with 1,2-dibromoethane at elevated temperatures, followed by treatment with $Me_3SiCl$) was used to form the initial organozinc species upon addition of the iodide under vigorous stirring. The first attempts in dry THF resulted in no reaction at all, but with DMF as a solvent the desired organozinc species were readily formed.

This species must then be transmetallated with copper cyanide. Upon mixing, copper(I) cyanide and lithium chloride form a green soluble complex within a few minutes. After three hours, stirring of the organozinc compound was discontinued and the zinc was allowed to settle. DMF was added to the CuCN/LiCl mixture and cooled to –18 °C, after which the organozinc reagent was added using a syringe. After five minutes, bromoacetylene **35** was added and the resulting mixture was allowed to warm to room temperature during the night. A successful conversion into the product could be readily observed in $^1$H–NMR since the newly formed methylene adjacent to the acetylene function shifts to δ 2.65 ppm, a rather unique position in most NMR-spectra. After several test reactions, the yield was optimized to 75% yield.
The addition of palladium catalysts has also been reported for these reactions, but in our hands did not change the outcome of the coupling. Furthermore, it has also been shown that the stoichiometric amounts of CuCN/2LiCl may be replaced by a catalytic amount of CuBr·SMe$_2$.[15] In this case, however, the catalytic procedure only led to decomposition.

## *3.4 New acetylenic amino acids*

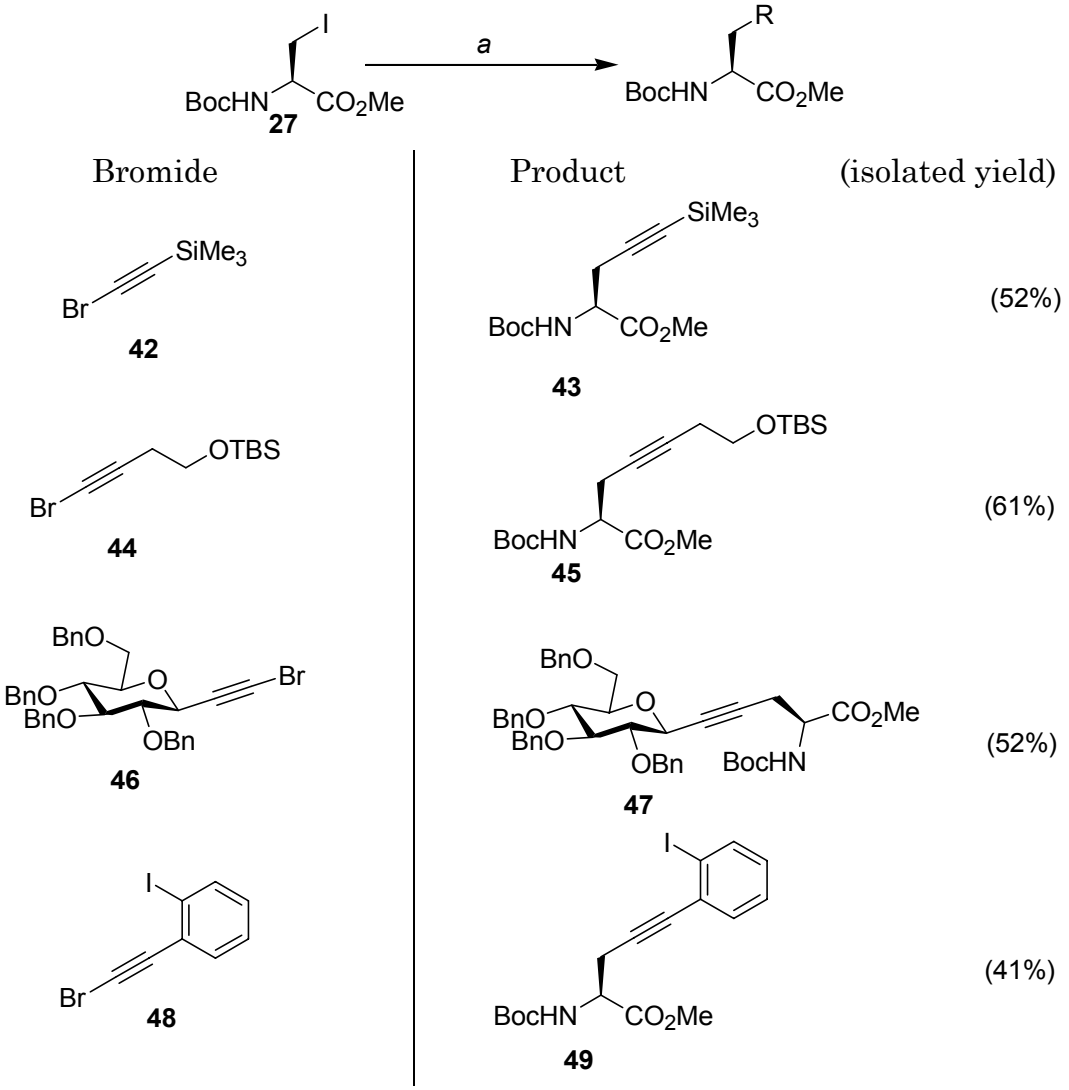
A small series of alkyl iodides was used to probe the viability of this method as shown in Scheme 12. In all cases, the desired products **38-40** were formed, with the primary iodides giving somewhat lower yields than the secondary iodide. In case the reaction was not carefully conducted under fully inert conditions, elimination was observed resulting in recovered bromoacetylene and the dehydroalanine derivative **41**.[16]




**Scheme 12**. *Reagents and conditions: a) Zn*, CuCN/2·LiCl, R-I, DMF.*

In summary, building block **35** can be used to provide a range of allyl functionalized acetylenic amino acids based on coupling of brominated 2-amino-4-pentynoic acid (**35**) with virtually any aliphatic iodide.

This first approach is somewhat hampered through the limited availability of 2-amino-4-pentynoic acid. In that sense, the alternative pathway, starting from readily available enantiopure serine, has a clear advantage. Thus, upon reaction of **27** with the required metals, coupling reactions with halogenated commercially available acetylenes were carried out (Scheme 13). For example, use of brominated $Me_3Si$-acetylene (**42**) yielded adduct **43** in 52% yield, which is a useful precursor for ring-closing alkyne metathesis (see: Chapter 5).[17]

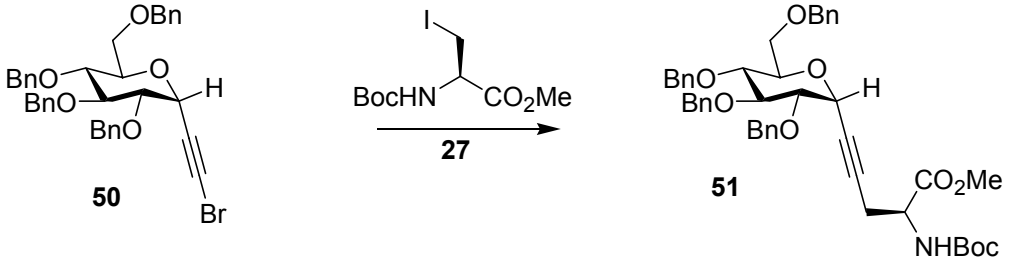



**Scheme 13**. *Reagents and conditions: a) Zn*, CuCN/2·LiCl, R-Br, DMF.*

Other interesting bromoacetylenes are sugar-derived acetylenes such as **46**. Coupling with **27** led to the glycoamino acid **47**, containing a stable linkage that might be isosteric with the naturally occurring labile acetal function. Considering the mild conditions, one could envision that this approach might be followed to make more complicated stable mimics of biologically relevant glycopeptides.[18] We proved that the optical purity was retained under these mild coupling conditions

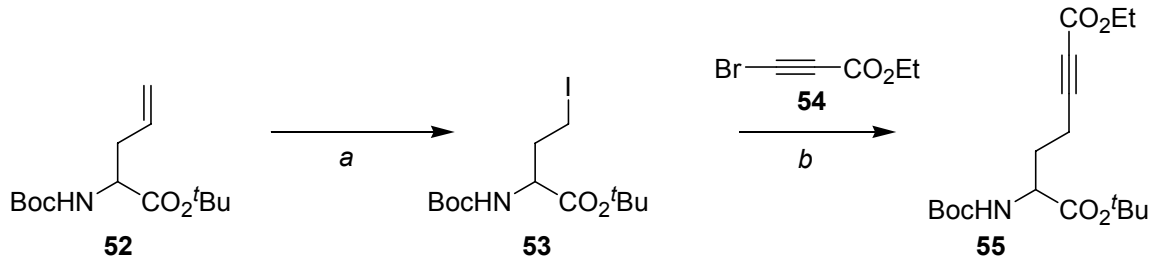
while previous methods involved strong basic conditions and amino acid protection to reach the same compounds.[19]

Interestingly, a similar approach was independently pursued in the group of Isobe (Scheme 14).[20] Describing an identical coupling of the same iodide with the α-configured acetylenic glucose derivative, the anomeric glycoamino acid **51** was obtained.




**Scheme 14**. *Reagents and conditions: a) Zn*, CuCN/2·LiCl, R-Br, DMF (40%).*

A fourth example involving bromide **54** led to the ester-substituted acetylenic amino acid **55**. In fact, one could consider **55** as a rigidified derivative of glutamic acid which could be used in the active site of peptide systems where glutamic acids usually display a dominant role.

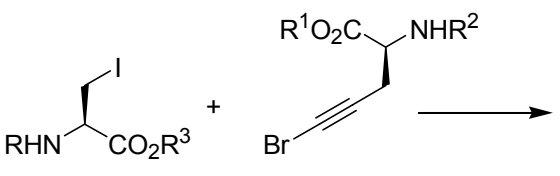



**Scheme 15**. *Reagents and conditions: a) i: $O_3$, $CH_2Cl_2$ then $NaBH_4$, MeOH; ii: $PPh_3$, $I_2$, imidazole, $CH_2Cl_2$ (20%, 2 steps); b) Zn*, CuCN/2·LiCl, **54**, DMF (27%).*

Sterically encumbered allylglycine *tert*-butyl ester (**52**) was transformed to homoserine via ozonolysis.[21,22] Treatment with triphenylphosphine and iodine provided the desired precursor **53** in a disappointing yield of 27%.[23] Using the optimized organozinc conditions, reaction with bromide **54** afforded product **55** in an isolated 27% yield.[24] Presumably **54** also can undergo Michael-addition, which may cause the low yield.

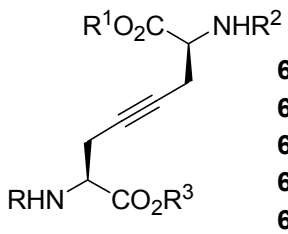
## *3.5 Direct isostere synthesis*

Having established a general protocol for the coupling of serine-derived iodides with bromoacetylenes, we focused our efforts on the formation of unsaturated diaminosuberic acid derivatives. Several differently protected iodides were made and coupled with orthogonally protected bromoacetylenes (Scheme 16).



| | R | $R^3$ |
|---|---|---|
| **27** | Boc | Me |
| **56** | Boc | Bn |
| **57** | Boc | Allyl |
| **58** | Fmoc | Allyl |
| **59** | Cbz | $^t$Bu |

| | $R^1$ | $R^2$ |
|---|---|---|
| **35** | Me | Boc |
| **60** | Me | Ns |
| **61** | $^t$Bu | Fmoc |
| **62** | Bn | Fmoc |
| **63** | $Ph_2C$= | Me |



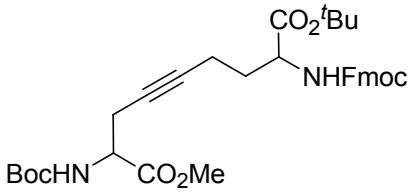
| | R | $R^3$ | $R^2$ | $R^1$ | yield (%) |
|---|---|---|---|---|---|
| **64** | Boc | Me | Boc | Me | 61 |
| **65** | Boc | Me | $Ph_2C$= | Me | 43 |
| **66** | Boc | Me | Ns | Me | - |
| **67** | Trt | Me | Boc | Me | - |
| **68** | Cbz | $^t$Bu | Fmoc | Bn | - |
| **69** | Cbz | $^t$Bu | Boc | Me | - |
| **70** | Fmoc | Allyl | Boc | Me | - |
| **71** | Boc | Allyl | Boc | Me | 35 |
| **72** | Boc | Bn | Fmoc | Bn | - |
| **73** | Fmoc | Bn | Boc | Me | - |

**Scheme 16**. *Reagents and conditions: Zn*, CuCN/2·LiCl, DMF.*

Initially, the reactions were performed with *rac*-**35** derivatives and (*S*)-serine-derived iodides, both in *tert*-butyl carbamate- and methyl ester-protected form. This coupling under identical conditions eventually gave rise to a gratifying yield of 61% of **64** as a mixture of the two possible diastereoisomers. The non-orthogonal isostere **65** also resulted in a reasonable yield of 43%. This example has the main advantage that the propargyl unit can be made according to asymmetric protocols as outlined in chapter 2.

Attempts to utilize Ns- or Trt-groups (**66** and **67**) were unsuccessful due to the nature of the protecting group. Use of Cbz (**68** and **69**), Bn (**72** and **73**) and Fmoc (**68**, **70**, **72** and **73**) were also to no avail. In some cases, only unidentifiable side-products were formed in low yield. On the other hand, an allyl ester such as **71** could be coupled in 35% yield.

These attempts to find a generic protocol for orthogonal protection led us to conclude that this is not possible in a straightforward manner. Apart from the difficult syntheses and side-reactions that take place in a number of cases, yields are generally rather low when coupling two amino acid fragments using this methodology.




**74**

**Figure 3**.

Somewhat in contrast, in case a homoserine-derived iodide was used, a facile conversion to orthogonally protected **74** (Figure 3) was achieved in 59% yield, albeit as a mixture of all possible diastereoisomers.

## *3.6 Conclusions*

The investigations concerning organozinc couplings of acetylenes provided interesting pathways for the synthesis of new amino acids under mild, non-racemizing conditions. Since the amino acid moiety can either act as the electrophile or nucleophile, a wide range is open for its application. This is confirmed by several interesting examples which appeared in literature during the course of this research.
However, the ultimate goal of synthesizing fully orthogonally protected diaminosuburic acid derivatives proved to be difficult to reach. Despite these setbacks, a reaction based on a homoserine-derived iodide provided an example of such a molecule in satisfactory yield.

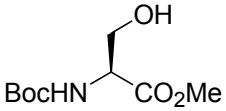
## *3.7 Acknowledgements*

Jasper Kaiser is kindly acknowledged for his work on bromide **54** and homoserine-derived iodide **74**. Bas van den Broek is kindly acknowledged for the provision of bromide **46**.

## *3.8 Experimental section*

For general experimental details, see: Section 2.8.

**Methyl 2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropanoate (31)**[25].

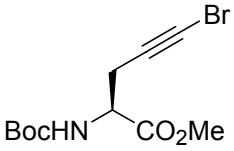



To a suspension of (*S*)-serine (2.00 g, 19.0 mmol) in MeOH (25 mL) was added dropwise $SOCl_2$ (4.00 mL, 52.8 mmol) and the resulting clear solution was refluxed for 2 h. The volatiles were

removed *in vacuo* and $Et_3N$ (6.00 mL, 42.7 mmol) in THF (50 mL) was added. To this solution $Boc_2O$ (5.92 g, 27.1 mmol, lumps in frozen form) was added and the resulting mixture was stirred overnight at room temperature. After evaporation further purification using flash chromatography (50% EtOAc in heptane) yielded **31** as a clean oil (4.13 g, 18.8 mmol, 99%). An alternative method uses $K_2CO_3$ (3.97 g, 26.4 mmol) and Boc-Ser-OH (4.92 g, 24.0 mmol) which were suspended in DMF (25 mL) at 0 °C and vigorously stirred. After 10 min, MeI (3.03 mL, 48.2 mmol) was added dropwise and stirring was continued for 2 h. Water (40 mL) was added and the reaction mixture was extracted using EtOAc (3 × 40 mL). The organic layer was washed (brine, 25 mL), dried and evaporated, yielding protected amino acid **31** (3.57 g, 16.1 mmol, 66%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.49−5.34 (bs, 1H, NH), 4.44−4.32 (m, 1H, CαH), 4.00−3.86 (m, 2H, C*H*$_2$OH), 3.78 (s, 3H, OMe), 2.37−2.22 (m, 1H, OH), 1.46 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 171.4, 155.7, 80.0, 63.1, 55.6, 52.4, 28.1.

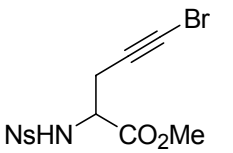
*General procedure for bromination:*

**Methyl (2*S*)-5-bromo-2-[(*tert*-butoxycarbonyl)amino]-4-pentynoate (35).**



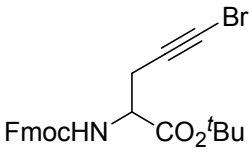

To a solution of *N*-bromosuccinimide (5.64 g, 31.5 mmol) and 2-*tert*-butoxycarbonylamino-4-pentynoic acid methyl ester (**34**,6.81 g, 31.4 mmol) in acetone (90 mL) was added $AgNO_3$ (10.0 mg, 0.06 mmol). The mixture was stirred for 1 h and monitored using TLC (Note: $R_f$-values differ ~ 0.02 or less). Water (100 mL) was added and the mixture was extracted using pentane (3 × 75 mL). The organic layers were combined and washed (brine, 50 mL), dried ($Na_2SO_4$) and evaporated, yielding **35** as a yellowish solid (7.20 g, 25.5 mmol, 85%). $[\alpha]_D$ = +39.4 (*c* = 1.46, $CH_2Cl_2$); Mp = 55 °C; $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.35 (d, *J* = 7.7 Hz, 1H, NH), 4.47−4.32 (m, 1H, $C_\alpha$H), 3.76 (s, 3H, OMe), 2.73 (d, *J* = 5.0 Hz, 2H, $CH_2$), 1.44 (s, 9H, Boc); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 171.0, 155.0, 80.3, 74.6, 52.7, 51.9, 41.7 (C≡*C*Br), 28.3, 24.1; IR ν 2977, 1747, 1714, 1507, 1366, 1248, 1163, 1058 cm$^{-1}$; HRMS (EI): calculated for $C_7H_8NO_4Br$ $(M - Boc)^+$, 248.96367, found 248.96357.

**Methyl 5-bromo-2-[(4-nitrophenyl)sulfonyl]amino-4-pentynoate (60).**

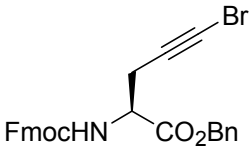



Using the general procedure for bromination 2-[4-nitrophenylsulfonylamino]-4-pentynoic acid methyl ester (347 mg, 1.11 mmol) was transformed in its corresponding bromide **60** (427 mg, 1.09 mmol, 98%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 8.06−8.03 (m, 1H, ArH), 7.91−7.87 (m, 1H, ArH), 7.74−7.71 (m, 2H, 2 × ArH), 6.40 (bd, *J* = 8.7

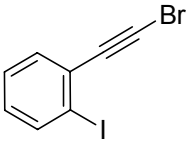
Hz, 1H, NH), 4.34−4.28 (m, 1H, CαH), 3.57 (s, 3H, OMe), 2.76 (d, *J* = 5.1 Hz, 2H, C≡CCH₂); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 169.5, 133.8, 133.0, 130.2, 125.6, 73.1, 54.8, 53.0, 29.5, 24.8; IR ν 3305, 3094, 2955, 1742, 1708, 1538, 1355, 1167 cm$^{-1}$.

***tert*-Butyl (2*S*)-5-bromo-2-[(9*H*-9-fluorenylmethoxy)carbonyl]amino-4-pentynoate (61).** 

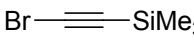
Using the general procedure for bromination 2-(9-fluorenyl-methoxycarbonyl)amino-4-pentynoic acid *tert*-butyl ester (371 mg, 0.95 mmol) was brominated, resulting in **61** as a white solid (358 mg, 0.76 mmol, 81%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H, 2 × ArH), 7.58 (d, *J* = 7.5 Hz, 2H, 2 × ArH), 7.37 (t, *J* = 7.2 Hz, 2H, 2 × ArH), 7.29 (t, *J* = 7.2 Hz, 2H, 2 × ArH), 5.65 (bd, *J* = 7.5 Hz, 1H, NH), 4.43−4.33 (m, 3H, $OCH_2$, CαH), 4.22 (t, *J* = 6.9 Hz, 1H, FmocαH), 2.76 (d, *J* = 4.2 Hz, 2H, C≡C$CH_2$), 1.49 (s, 9H, Boc); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 168.8, 155,2, 143.5, 141.0, 127.5, 126.9, 124.9, 119.8, 82.9, 74.8, 67.2, 52.8, 47.2, 28.1, 24.2.

**Benzyl (2*S*)-5-bromo-2-[(9*H*-9-fluorenylmethoxy)carbonyl]amino-4-pentynoate (62).** 

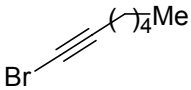
(*S*)-2-(9-fluorenyl-methoxycarbonyl)amino-4-pentynoic acid (594 mg, 1.77 mmol), dissolved in DMF (15 mL) was treated with $K_2CO_3$ (273 mg, 1.95 mmol) and benzyl bromide (362 mg, 2.12 mmol) and stirred overnight. After partitioning between $H_2O$ (30 mL) and heptane (40 mL) the aqueous phase was extracted with heptane (2 × 30 mL). The combined organic layers were washed (brine, 20 mL), dried ($Na_2SO_4$) and evaporated which yielded 310 mg of the benzyl ester. Using the general method for bromination this solid was transformed into its corresponding bromide **62**, a white solid (287 mg, 0.61 mmol, 35%, 2 steps). $[\alpha]_D$ = 7.4 (*c* = 1.15, $CH_2Cl_2$); $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.3 Hz, 2H, ArH-Fmoc), 7.61 (d, *J* = 7.3 Hz, 2H, ArH-Fmoc), 7.43−7.31 (m, 9H, 5 × OBn, 4 × ArH-Fmoc), 5.65 (bd, *J* = 8.7 Hz, 1H, NH), 5.24 (q, *J* = 15.1 Hz, 2H, Ar$CH_2$O), 4.48−4.52 (m, 1H, CαH), 4.43−4.38 (m, 2H, $CH_2$O-Fmoc), 4.25 (t, *J* = 7.3 Hz, 1H, CαHFmoc), 2.83 (d, *J* = 4.4 Hz, $CH_2$, BrC≡C$CH_2$); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 170.0, 155.4, 143.7, 141.1, 134.9, 128.5, 128.4, 128.2, 127.6, 127.0, 125.0, 119.9, 74.4, 67.5, 67.1, 52.4, 46.9, 23.8; IR ν 2977, 1747, 1714, 1506, 1366, 1248, 1163, 1068 cm$^{-1}$; HMRS (FAB)$^+$ calcd for $C_{27}H_{23}O_4NBr$ 504.08105, found 504.0810.



**1-(2-Bromo-1-ethynyl)-2-iodobenzene (48).**[25] To a solution of phenylacetylene (1.50 g, 14.9 mmol) in THF (15 mL) was added, at below −40 °C, butyllithium (16.5 mL, 2.2 M in hexane). The mixture of lithium phenylacetylene and butyllithium thus obtained was cooled to −70 °C and a solution of $^t$BuOK (3.2 g, 28 mmol) in THF (20 mL) was added over 30 min, while maintaining the temperature of −70 °C. After stirring for an additional period of 30 min at this temperature, the cooling system was removed. The temperature was allowed to rise to 5 °C, after which a further amount of $^t$BuOK (1.70 g, 15 mmol) in THF (10 mL) was added to destroy any unreacted alkyllithium. A fresh solution of $MgBr_2$ (obtained by slowly adding 1,2-dibromoethane (9.81 g, 52.2 mmol) to a stirred suspension of magnesium (1.82 g, 67 mmol) and 15 mL of refluxing diethyl ether) was added at 0 °C and stirring at 25 °C was continued for 1 hour. Iodine (5.2 g, 21 mmol) was added and the reaction was quenched after 45 min by addition of a cold 2 M HCl solution (20 mL). The reaction mixture was extracted using pentane (3 × 30 mL), washed using aqueous saturated sodium thiosulfate, dried ($Na_2SO_4$) and evaporated. Further purification using column chromatography (2% EtOAc in heptane) afforded the desired compound (2.83 g, 1.24 mmol, 67%) as yellowish oil. $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.84 (dt, *J* = 1.2 Hz, 8.1 Hz, 1H, IC=C*H*), 7.46 (dq, *J* = 1.8 Hz, 7.8 Hz, 1H, Ar), 7.30 (dt, *J* = 1.2 Hz, 7.5 Hz, 1H, Ar), 7.02 (dq, *J* = 1.5 Hz, 7.5 Hz, 1H, Ar), 3.39 (s, 1H, C≡CH); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 138.7, 138.5, 133.4, 129.9, 127.7, 127.6, 100.4, 80.9. Using the general method for bromination 1-ethynyl-2-iodo-benzene (1.01 g, 4.45 mmol) was transformed into its corresponding bromide **48** (1.179 g, 3.85 mmol, 86%) as an oil. $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.81 (dd, *J* = 0.9 Hz, 8.1 Hz, 1H, IC=C*H*), 7.43 (dd, *J* = 1.2 Hz, 7.2 Hz, 1H), 7.28 (dt, *J* = 1.2 Hz, 7.5 Hz, 1H), 7.01 (dt, *J* = 1.5 Hz, 7.8 Hz, 1H); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 138.6, 138.5, 133.5, 133.2, 129.7, 1277, 100.5, 54.1 (*C*Br).



**(2-Bromo-1-ethynyl)(trimethyl)silane (42).**[25] Using the general procedure for bromination (1-ethynyl)(trimethyl)silane (1.00 g, 10.2 mmol) was transformed, resulting after distillation in product **42** (1.14 g, 6.4 mmol, 63%). $^1$H−NMR ($CDCl_3$, 300 MHz) δ 0.19 (s, 9H, $SiMe_3$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 104.0, 41.9, -0.2.

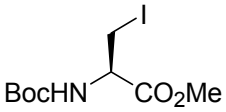


**1-Bromo-1-heptyne.**[25] Using the general procedure for bromination 1-heptyne (1.50 g, 15.0 mmol) was transformed to its

corresponding bromide (2.592 g, 14.8 mmol, 99%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 2.20 (t, *J* = 7.0 Hz, 2H, C≡C$CH_2$), 1.54−1.27 (m, 6H, 3 × $CH_2$), 0.90 (t, *J* = 7.0 Hz, 3H, Me); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 80.3, 38.6 (CBr), 32.7, 29.5, 21.3, 20.6, 15.0.

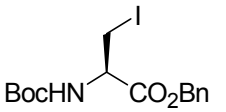
*General procedure for iodination:*

**Methyl (2*R*)-3-iodo-2-[(*tert*-butoxycarbonyl)amino]propanoate (27).**[25]



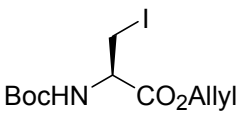

To a stirred solution of Boc-Ser-OMe (**30**, 5.50 g, 25.0 mmol) in $CH_2Cl_2$ (100 mL) were added iodine (7.00 g, 27.6 mmol), imidazole (1.88 g, 27.6 mmol) and $PPh_3$ (7.70 g, 29.4 mmol) and the resulting yellow solution was stirred overnight. After evaporation of the volatiles compound **27** was isolated using flash chromatography (20% EtOAc in heptane), yielding the desired product as white solid (5.94 g, 18.1 mmol, 72%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 5.38 (bs, 1H, NH), 4.46−4.41 (m, 1H, CαH), 3.81 (s, 3H, OMe), 3.61−3.55 (m, 2H, $CH_2$I), 1.44 (s, 9H, Boc); $^{13}$C (75.5 MHz, $CDCl_3$) δ 171.9, 157.2, 81.0, 58.6, 57.0, 30.0, 8.1.

**Benzyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate (56).**[25]

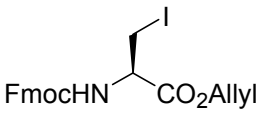



Using the general procedure Boc-Ser-OBn (500 mg, 1.69 mmol) was transformed, yielding **56** as a white solid (380 mg, 0.94 mmol, 55%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 5H, Ar), 5.45 (bd, *J* = 7.3 Hz, 1H, NH), 5.29 (d, *J* = 1.9 Hz, 2H, O$CH_2$Ar), 4.67−4.59 (m, 1H, CαH), 3.66 (t, *J* = 3.8 Hz, 2H, $CH_2$I), 1.53 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 169.0, 154.4, 134.5, 128.3 (2 ×), 105.9, 80.4, 67.9, 53.7, 28.3, 7.6.

**Allyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate (57).**

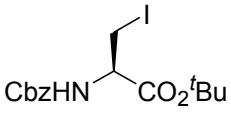



Using the general procedure Boc-Ser-Allyl (753 mg, 3.10 mmol) was iodinated and isolated as white solid **57** (697 mg, 1.96 mmol, 64%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.93 (ddt, *J* = 6.8 Hz, 21.4 Hz, 1H, C=CH), 5.37 (app dq, *J* = 1.5 Hz, 17.1 Hz, 1H, C=$CH_2$), 5.27 (app dq, *J* = 1.2 Hz, 10.6 Hz, 1H, C=$CH_2$), 5.35 (bs, 1H, NH), 4.74−4.61 (m, 2H, O$CH_2$), 4.54−4.50 (m, 1H, CαH), 3.63−3.53 (m, 2H, $CH_2$I), 1.45 (s, 9H, Boc); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 169.2, 154.8, 131.2, 119.3, 80.4, 66.6, 53.5, 28.2, 7.7; IR ν 2924.1, 2852.4, 1716.0, 1497.0, 1163.8 $cm^{-1}$.

**Allyl 2*R*)-2-[(9*H*-9-fluorenylmethoxy)carbony]amino-3-iodopropanoate (58).** 

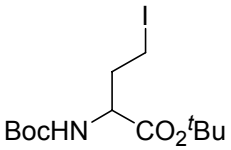
Using the general procedure Fmoc-Ser-Allyl was converted to its corresponding iodide **58**, which was obtained as white crystals (618 mg, 1.32 mmol, 70%). $[\alpha]_D$ = +9.6 (*c* = 0.23, $CH_2Cl_2$); $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H, 2 × ArH), 7.62 (d, *J* = 7.5 Hz, 2H, 2 × ArH), 7.41 (t, *J* = 7.2 Hz, 2H, 2 × ArH), 7.36−7.30 (m, 2H, 2 × ArH), 6.00−5.88 (m, 1H, CH=C), 5.67 (bd, *J* = 7.2 Hz, 1H, NH), 5.41−5.30 (m, 2H, C=$CH_2$), 4.71 (d, *J* = 5.4 Hz, 2H, AllylC*H*$_2$O), 4.64−4.58 (m ,1H, CαH), 4.48−4.35 (m, 2H, $CH_2O$), 4.26 (t, *J* = 7.2 Hz, 1H, CH-Fmoc), 3.63 (d, *J* = 3.6 Hz, 2H, $CH_2I$); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 169.0, 154.9, 143.7, 141.3, 131.1, 127.8, 127.1, 125.1, 120.0, 119.6, 67.4, 66.9, 54.0, 47.1, 7.6; IR v 3406.4, 3063.5, 2948.5, 1710.6, 1515.7, 1448.4, 1313.7, 1187 cm$^{-1}$; HRMS (FAB)$^+$ calcd for $C_{21}H_{21}O_4NI$ 478.05153, found 478.0515.

***tert*-Butyl (2*R*)-2-[(benzyloxy)carbonyl]amino-3-iodopropanoate (59).**[25]



A mixture of DCC (16.5 g, 80.0 mmol), $^t$BuOH (7.60 g, 105 mmol) and copper-(I)-chloride (150 mg, 1.49 mmol) was stirred for 3 days.[26] The green suspension was then diluted with dry $CH_2Cl_2$ (20 ml) and the *N*-protected amino acid (6.00 g, 25.1 mmol) in $CH_2Cl_2$ (30 ml) was added dropwise. The reaction was finished in 1 hour. Precipitated urea was then removed by filtration. The organic layer was washed using saturated $NaHCO_3$ solution (3 × 50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Crude NMR revealed no further purification was necessary $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.37−7.30 (m, 5H, Ar), 5.65 (bs, 1H, NH), 5.12 (s, 2H, Ar$CH_2O$), 4.36−4.29 (m, 2H, CαH), 3.93 (s, 2H, $CH_2OH$), 2.21 (bs, 1H, OH), 1.48 (s, 9H, $CMe_3$). Using the general protocol for iodination the crude amino acid was transformed to product **59**, an offwhite solid (9.05 g, 22.4 mmol, 89%, 2 steps). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.37−7.28 (m, 5H, Ar), 5.60 (bd, *J* = 7 Hz, 1H, NH), 5.14 and 5.13 (AB, *J* = 12 Hz, 2H, Ar$CH_2O$), 4.41 (dt, *J* = 3.5 Hz, 7 Hz, 1H, CαH), 3.59 (d, *J* = 3.5 Hz, 2H, $CH_2I$), 1.50 (s, 9H, $CMe_3$); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 168.1, 155.4, 136.1, 128.6, 128.1, 83.6, 67.1, 54.0, 28.0, 8.4.

***tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-4-iodobutanoate (53).**[25]

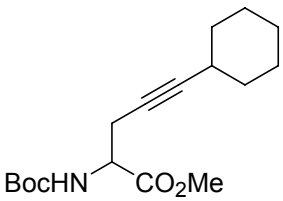


A solution of 2-*tert*-butoxycarbonylamino pent-4-enoic acid *tert*-butyl ester (**52**, 934 mg, 3.40 mmol) was dissolved in $CH_2Cl_2$ (50 mL) and cooled to −78 °C. $O_3$ was bubbled through the solution and when a blue color remained visible, the ozone-generator was

turned off allowing oxygen to remove traces of ozone. $NaBH_4$ (169 mg, 4.60 mmol) and MeOH (5 mL) were added and the temperature was allowed to warm to room temperature. The mixture was quenched with water (15 mL) and extracted with $CH_2Cl_2$ (2 × 30 mL), the combined organic layers were washed (brine), dried ($MgSO_4$) and evaporated. Further purification using flash chromatography (25% EtOAc in heptane) yielded the desired alcohol (257 mg, 0.93 mmol) in 27%. $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.32 (d, *J* = 6.9 Hz, 1H, NH), 3.62 (m, 2H, C*H*$_2$OH), 2.06 (m, 1H, $CH_2$), 1.50 (m, 1H, $CH_2$), 1.40 (s, 9H, Boc), 1.38 (s, 9H, $CMe_3$); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 172.2, 156.8, 82.5, 80.5, 58.4, 51.3, 36.7, 28.5, 28.2. Using general iodination conditions the amino acid (257 mg, 0.93 mmol) was transformed to its corresponding iodide **53** (250 mg, 0.88 mmol, 69.5%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.08−5.01 (m, 1H, NH), 4.22−4.12 (m, 1H, CαH), 3.20−3.10 (m, 2H, $CH_2I$), 2.44−2.29 (m, 1H, $CH_2$), 2.22−2.05 (m, 1H, $CH_2$), 1.47 (s, 9H, Boc), 1.44 (s, 9H, $CMe_3$); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 169.0, 154.9, 82.4, 82.3, 54.9, 37.8, 28.3, 27.9, 0.57.

*General procedure for organozinc couplings*

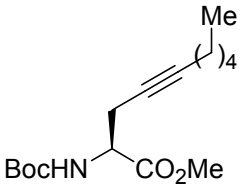
**Methyl 2-[(*tert*-butoxycarbonyl)amino]-5-cyclohexyl-4-pentynoate (40).**




Zinc dust (116 mg, 1.408 mmol) was weighed into a 20 mL flask, which was repeatedly evacuated (with heating using a heat gun) and flushed with argon. Dry DMF (0.5 mL, distilled from $CaH_2$) and 1,2-dibromoethane (9.2 μL, 0.106 mmol) were added and the flask was heated at 80 °C for 40 min. The reaction mixture was allowed to cool to room temperature, trimethylsilyl chloride (4 μL, 0.035 mmol) was added and the resulting mixture was stirred vigorously for a further 30 min under argon. Iodocyclohexane (69 μl, 0.528 mmol) was added and stirred at room temperature for 3 h more after which stirring was ceased to settle the zinc. CuCN (41 mg, 0.46 mmol) and LiCl (40 mg, 0.92 mmol) were heated to 150 °C for 2 h and cooled to room temperature. Addition of DMF (1 mL) formed a soluble CuCN·2LiCl complex within 5 min. After cooling the Cu-complex to –15 °C, the organozinc reagent was added dropwise followed by bromoacetylene (*rac*)-**35** (116 mg, 0.35 mmol). The mixture was allowed to stir overnight at room temperature. Water was added and the suspension was extracted using heptane, washed (brine), dried ($MgSO_4$) and concentrated. Purification using flash column chromatography (10% EtOAc in heptane) yielded **40** (100 mg, 81%) as a colorless oil. $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.28 (d, *J* = 7.7 Hz, 1H, NH), 4.43−4.38 (m, 1H, CαH), 3.73 (s, 3H, OMe),

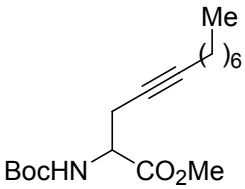
2.69–2.63 (m, 2H, C≡CCH$_2$), 2.13 (m, 1H, CC≡CH), 1.73–1.22 (m, 10H, 5 × CH$_2$), 1.43 (s, 9H, Boc); $^{13}$C–NMR (75 MHz, CDCl$_3$) δ 171.4, 155.0, 88.1, 79.9, 73.8, 52.3, 32.7, 32.7, 28.8, 28.2, 25.8, 24.6, 23.1; IR ν 3355, 2929, 2852, 2359, 2337, 1749, 1717, 1498, 1447, 1365, 1251, 1181, 1060 cm$^{-1}$; HRMS (EI): calculated for $C_{17}H_{27}NO_4$ 309.1940, found 309.1937.

**Methyl 2*S*-[(*tert*-butoxycarbonyl)amino]-4-decynoate (36).**



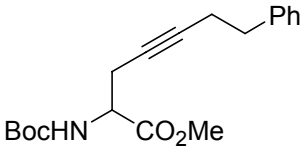

Using the general method of zincactivation, Serine derived iodide **27** (125 mg, 0.37 mmol) was treated with 1-bromo-1-heptyne (66 mg, 0.38 mmol). Purification using flash chromatography (25% EtOAc in heptane) yielded product **36** (68 mg, 0.27 mmol, 60%) as an oil. $^1$H–NMR (300 MHz, CDCl$_3$) δ 5.27 (bd, 1H), 4.41 (m, 1H), 3.75 (s, 3H), 2.69–2.64 (m, 2H), 2.13–2.09 (m, 2H), 1.46 (s, 9H), 1.40–1.18 (m, 8H), 0.87 (t, *J* = 6.9 Hz, 3H).

**Methyl 2*S*-[(*tert*-butoxycarbonyl)amino]-4-dodecynoate (37).**



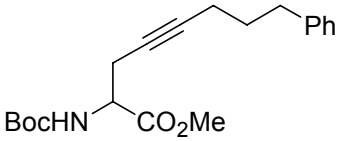

Using the general method of zincactivation, 1-iodide heptane (177 mg, 0.79 mmol) was treated with (*rac*)-**35** (220 mg, 0.719 mmol). Purification using flash chromatography (25% EtOAc in heptane) yielded product **37** (134 mg, 0.41 mmol) in 57%. $^1$H–NMR (400 MHz, CDCl$_3$) δ 5.28 (bd, *J* = 8.3 Hz, 1H, NH), 4.44–4.38 (m, 1H, CαH), 3.74 (s, 3H, OMe), 2.76–2.58 (m, 2H, CHC*H$_2$*C≡C), 2.14–2.09 (m, 2H, C≡CCH$_2$), 1.44 (s, 9H, Boc), 1.33–1.21 (m, 8H, aliphatic chain), 0.87 (t, *J* = 6.8 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, CDCl$_3$) δ 171.5, 155.1, 84.0, 80.0, 73.8, 52.4, 52.3, 31.7, 28.8, 28.7, 28.3, 23.1, 22.6, 18.6, 14.0; IR ν 2927, 2856, 1747, 1714, 1501, 1365, 1165 cm$^{-1}$; HMRS (FAB)$^+$ calcd for $C_{18}H_{32}O_4N$ 326.23313, found 326.2331.

**2-*tert*-Butoxycarbonylamino-7-phenyl-hept-4-ynoic acid methyl ester (38).**



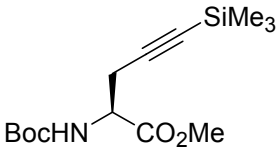

Following the general procedure for organozinc coupling, phenethyl iodide (233 mg, 1.00 mmol) was combined with (*rac*)-**35** (212 mg, 0.69 mmol) to result in **38** as an amorphous solid (90 mg, 0.27 mmol, 39%). $^1$H–NMR (300 MHz, CDCl$_3$) δ 7.35–7.15 (m, 5H, Ar), 5.25–5.20 (m, 1H, NH), 4.45–4.40 (m, 1H, CαH), 3.73 (s, 3H, OMe), 2.99–2.93 (m, 2H, C≡CCH$_2$), 2.80–2.32 (m, 4H, 2 × CH$_2$), 1.47 (s, 9H, Boc); $^{13}$C–NMR (75 MHz, CDCl$_3$) δ 171.4, 155.1, 140.6, 128.8, 128.4, 126.2, 83.0, 79.9, 74.8, 52.4, 52.2, 35.0, 28.3, 23.0, 20.8; EI-MS *m*/*z*: 331 (M)$^+$.

**2-*tert*-Butoxycarbonylamino-8-phenyl-oct-4-ynoic acid methyl ester (39).**



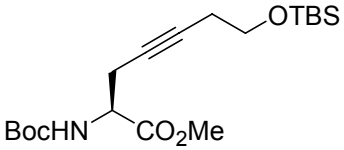

Using the general procedure for organozinc coupling 1-iodo-3-phenylpropane (267 mg, 1.08 mmol) was combined with (*rac*)-**35** (217 mg, 0.71 mmol) to yield **39** as an amorphous solid (72 mg, 0.21 mmol, 29%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.35−7.15 (m, 5H, Ar), 5.34 (bd, *J* = 7.6 Hz, 1H, NH), 4.47−4.41 (m, 1H, CαH), 3.76 (s, 3H, OMe), 2.79−2.67 (m, 4H, C≡C$CH_2$, $CH_2$Ar), 2.20−2.10 (m, 2H, C≡C$CH_2$), 1.81−1.74 (m, 2H, $CH_2$), 1.46 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 171.5, 155.1, 141.5, 128.4, 128.3, 125.8, 83.3, 80.0, 74.5, 52.4, 52.3, 34.3, 30.3, 28.2, 23.1, 18.0; EI-MS *m/z*: 345 $(M)^+$.

**Methyl 2*S*-[(*tert*-butoxycarbonyl)amino]-5-(1,1,1-trimethylsilyl)-4-pentynoate[27] (43).**




Using the general method of zincactivation, Serine derived iodide **27** (200 mg, 0.60 mmol) was treated with bromoethynyl-trimethyl-silane (215 mg, 1.2 mmol). Purification using flash chromatography (25% EtOAc in heptane) yielded product **43** (95 mg, 0.32 mmol, 52%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.29 (bd, *J* = 8 Hz, 1H, NH), 4.48−4.46 (m, 1H, CαH), 3.77 (s, 3H, OMe), 2.78 (dd, *J* = 5 Hz, 17 Hz, 1H, C≡C$CH_2$), 2.69 (dd, *J* = 5 Hz, 17 Hz, 1H, C≡C$CH_2$), 1.46 (s, 9H, Boc), 0.14 (s, 9H, $SiMe_3$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 171.3, 155.2, 127.6, 100.8, 80.1, 52.4, 52.1, 28.2, 24.2, 0.1.

**Methyl 2*S*-[(*tert*-butoxycarbonyl)amino]-7-[1-(*tert*-butyl)-1,1-dimethylsilyl]oxy-4-heptynoate (45).**



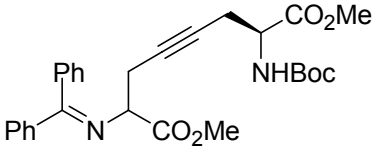

Using the general method of zincactivation, Serine derived iodide **27** (400 mg, 1.20 mmol) was treated with (4-bromo-but-3-ynyloxy)-tert-butyl-dimethyl-silane (266 mg, 1.01 mmol). Purification using flash chromatography (25% EtOAc in heptane) yielded product **45** (231 mg, 0.60 mmol, 59%) as an oil. $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.28 (bd, *J* = 9.3 Hz, 1H, NH), 4.45−4.38 (m, 1H, CαH), 3.75 (s, 3H, OMe), 3.66 (t, *J* = 7.2 Hz, 2H, $CH_2O$), 2.77−2.57 (m, 2H, C≡C$CH_2$Cα), 2.39−2.29 (m, 2H, C≡C$CH_2CH_2O$), 1.45 (s, 9H, Boc), 0.88 (s, 9H, $CMe_3$), 0.06 (s, 6H, 2 × SiMe); $^{13}$C−NMR (75 Mhz, $CDCl_3$) δ 171.1, 155.1, 100.7, 88.1, 80.1, 52.7, 52.1, 28.3, 28.2, 25.8, 23.8, 18.6, −5.3; IR ν 3416, 2976, 1746, 1712, 1498, 1161 $cm^{-1}$; EI-MS *m/z*: 606 $(M)^+$ 386, 312, 272, 228, 168, 94, 89.

**Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-5-(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-tri(benzyloxy)-6-[(benzyloxy)methyl]tetrahydro-2*H*-2-pyranyl-4-pentynoate (47).** Using the general procedure for zinccouplings, sugar-moiety **46** (73 mg, 0.14 mmol) was coupled with **27** (140 mg, 0.42 mmol) to deliver **47** as a white solid (54 mg, 0.07 mmol, 52%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.38−7.25 (m, 18H, ArH), 7.14−7.12 (m, 2H, ArH), 5.31 (d, *J* = 8.7 Hz, 1H, NHBoc), 4.96 (d, *J* = 10.7 Hz, 1H), 4.89 (d, *J* = 11.0 Hz, 1H), 4.82 (d, *J* = 4.9 Hz, 1H), 4.80 (s, 1H), 4.78 (d, *J* = 4.9 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.52 (d, *J* = 10.8 Hz, 1H), 4.49−4.44 (m, 1H, CαH), 4.02 (d, *J* = 9.1 Hz, 1H), 3.74 (dd, *J* = 1.9 Hz, 11.9 Hz, 1H), 3.67 (s, 3H, OMe), 3.62−3.54 (m, 3H), 3.44−3.40 (m, 1H), 2.87−2.77 (m, 2H, C≡C$CH_2$), 1.42 (s, 9H, Boc); $^{13}$C−NMR (75 Mhz, $CDCl_3$) δ 171.0, 154.2, 138.4, 138.0, 137.9, 137.8, 128.7, 127.9, 127.8 (2 ×), 127.6, 127.5, 127.3, 85.9, 82.2, 80.9, 80.8, 80.6, 78.8, 77.5, 77.1, 76.5, 75.6, 75.2, 74.9, 73.4, 69.8, 68.6, 28.2, 27.8, 23.2; IR ν 3353, 3031, 2876, 2240, 1749, 1718, 1455, 1089 cm$^{-1}$; MS (FAB): *m*/*z* 750 $(M + H)^+$.

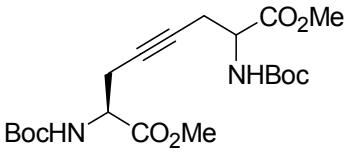
**Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-5-(2-iodophenyl)-4-pentynoate (49).** Using the general procedure for organozinc couplings **27** (643 mg, 1.96 mmol) was coupled with 1-(2-Bromo-1-ethynyl)-2-iodobenzene **48** (643 mg, 1.62 mmol) resulting in **49** as an amorphous solid (293 mg, 0.68 mmol, 42%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 1H, ArH), 7.39 (d, *J* = 7.7 Hz, 1H, ArH), 7.27 (t, *J* = 7.9 Hz, 1H, ArH), 6.97 (t, *J* = 7.6 Hz, 1H, ArH), 5.57 (bd, *J* = 8.1 Hz, 1H, NHBoc), 4.60−4.54 (m, 1H, CαH), 3.79 (s, 3H, OMe), 3.08−2.93 (m, 2H, C≡C$CH_2$), 1.44 (s, 9H, Boc); IR ν 2950.6, 2929.4, 2888.9, 1747.2, 1714.4, 1504.2, 1367.3, 1249.7, 1160.9, 1101.2, 1056.8, 837.0 cm$^{-1}$; HRMS (CI): calculated for $C_{17}H_{20}NO_4I$ 430.04370, found 430.03937.

**7-(*tert*-Butyl) 1-ethyl 6-[(*tert*-butoxycarbonyl)amino]-2-heptynedioate (55).** Using the general method of zincactivation, homoserine derived iodide **53** (112 mg, 0.29 mmol) was treated with bromo-propynoic acid ethyl ester (**54**,66.7 mg, 0.377 mmol). Purification using flash chromatography yielded **55** (26.0 mg, 0.073 mmol, 28%) as an oil. $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.09−4.99 (m, 1H, NH), 4.19 (q, *J* = 7.2 Hz, 2H, $CH_2O$), 2.43−2.36 (m, 2H, C≡C$CH_2$), 2.17−2.06 (m, 1H, $CH_2$), 1.95−1.81 (m, 1H, $CH_2$), 1.46 (s, 9H, Boc), 1.43 (s, 9H, $CMe_3$), 1.29 (t, *J*

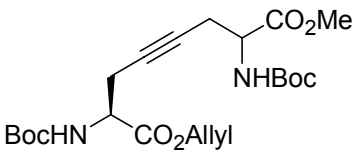
= 7.1 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 170.1, 155.3, 153.6, 87.7, 82.3, 80.9, 73.4, 81.8, 53.3, 31.1, 28.3, 27.9, 15.2, 14.0; IR ν 3376, 2977, 2236, 1712, 1504, 1367, 1253, 1154 cm$^{-1}$; HRMS (CI) calculated for $C_{18}H_{30}NO_6$ 356.20737, found 356.20741 $(M + H)^+$.

**Dimethyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-7-[(diphenylmethylene) amino]-4-octynedioate (65).** 

Using the general method of zinc-activation, **27** (250 mg, 0.76 mmol) was coupled with bromide **63** resulting in **65** (158 mg, 0.32 mmol, 43%). $^1$H–NMR (300 MHz, $CDCl_3$) δ 7.65-7.23 (m, 5H, Ar), 5.33-5.26 (m, 1H, NH), 4.40-4.34 (m, 1H, Cα*H*NH), 4.28 (dd, *J* = 5.4 Hz 8.0 Hz, 1H, CαH), 3.73 (s, 3H, OMe), 3.63 (d, *J* = 5.1 Hz (diasteromers), 3H, OMe), 2.88–2.60 (m, 4H, 2 × C≡C$CH_2$), 1.44 (s, 9H, Boc).

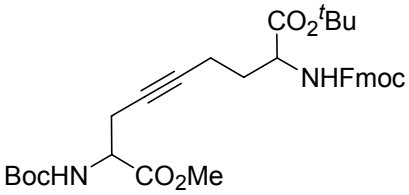
**Dimethyl (2*S*)-2,7-di[(*tert*-butoxycarbonyl)amino]-4-octynedioate (64).** 

Using the general method of zinc-activation, **27** (750 mg, 2.27 mmol) was coupled with **35** (581 mg, 1.90 mmol). After flash chromatography (40% EtOAc in heptane), product **64** was obtained as a sticky oil (497 mg, 1.16 mmol, 61%). $^1$H–NMR (300 MHz, $CDCl_3$) δ 5.62 (bd, *J* = 8.6 Hz, 1H, NH), 5.43 (bd, *J* = 7.6 Hz, 1H, NH), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 2.73–2.57 (m, 4H, C≡C$CH_2$), 1.44 (s, 18H, 2 × Boc); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 171.5, 171.3, 155.0 (2 ×), 80.0, 79.9, 77.9 (C≡), 52.5, 52.0, 28.2, 28.1, 23.4, 23.1; IR ν 3381.5, 2976.0, 1745.2, 1713.5, 1506.0, 1365.6, 1164.5, 1058.6 cm$^{-1}$.

**1-Allyl 8-methyl (2*S*)-2,7-di[(*tert*-butoxycarbonyl)amino]-4-octynedioate (71).** 

Using the general method of zinc-activation, **57** (250 mg, 0.703 mmol) was reacted with **35** (237 mg, 0.775 mmol). After purification using flash chromatography (25% EtOAc in heptane) product **71** was isolated as a clear sticky oil (113 mg, 0.25 mmol, 35%). $^1$H–NMR (300 MHz, $CDCl_3$) δ 5.92–5.80 (m, 1H, C=CH), 5.60 (m, 1H, NH), 5.41 (m, 1H, NH), 5.35–5.17 (m, 2H, =$CH_2$), 4.66–4.62 (m, 2H, O$CH_2$), 4.41 (m, 2H, 2 × CαH), 3.75 and 3.73 (2 × s, 3H *diastereomers*, OMe), 2.68–2.55 (m, 4H, 2 × C≡C$CH_2$), 1.40 and 1.40 and 1.3772 (3 × s, 18H *diastereomers*); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 171.2, 170.4, 155.0, 131.5, 118.8, 79.7, 66.0, 55.5, 52.0, 28.2, 28.1, 23.3, 23.0; IR ν

3383.5, 2976.5, 1743.4, 1712.5, 1502.3, 1366.0, 1161.9 $cm^{-1}$; HMRS $(FAB)^+$ calcd for $C_{22}H_{35}O_8N_2$ 455.23934, found 455.2398.

**9-(*tert*-Butyl) 1-methyl 2-[(*tert*-butoxycarbonyl)amino]-8-[(9*H*-9-fluorenylmethoxy)carbonyl]amino-4-nonynedioate (74).** 

Using the general method of zinc-activation, homoserine derived iodide **53** (470 mg, 0.93 mmol) was treated with **35** (214 mg, 0.70 mmol). Purification using flash chromatography (20% EtOAc in heptane) yielded amorphous solid **74** (250 mg, 0.41 mmol, 59%). $^1H$−NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H, 2 × ArH), 7.61 (d, *J* = 7.5 Hz, 2H, 2 × ArH), 7.38 (t, *J* = 7.2 Hz, 2H, 2 × ArH), 7.31 (t, *J* = 7.2 Hz, 2H, 2 × ArH), 5.75−5.39 (m, 2H, 2 × NH, diastereoisomers), 4.46−4.33 (m, 5H, 2 × CαH, C*H*-Fmoc), 4.23 (t, *J* = 5.4 Hz, 2H, FmocC$H_2$O), 3.75 (s, 3H, OMe), 2.63−2.69 (m, 2H, C≡CC$H_2$Cα), 2.19−2.25 (m, C≡C$CH_2$), 2.09−1.97 (m, 1H, $CH_2$), 1.74−1.86 (m, 1H, $CH_2$), 1.48 (s, 9H, Boc), 1.43 (s, 9H, $CMe_3$); $^{13}C$−NMR (75 Mhz, $CDCl_3$), 171.4 (2 ×), 155.8 (2 ×), 143.9, 141.3, 127.7, 125.1, 119.9, 82.3 (2 ×), 77.2 (2 ×), 66.9, 53.7, 52.4, 52.3, 47.1, 32.0, 28.3, 28.0, 23.1, 15.2; IR ν 3342, 2978, 2250, 1714, 1504, 1157, 738 $cm^{-1}$; EI-MS *m/z*: 606 $(M)^+$.

## *3.9 References and notes*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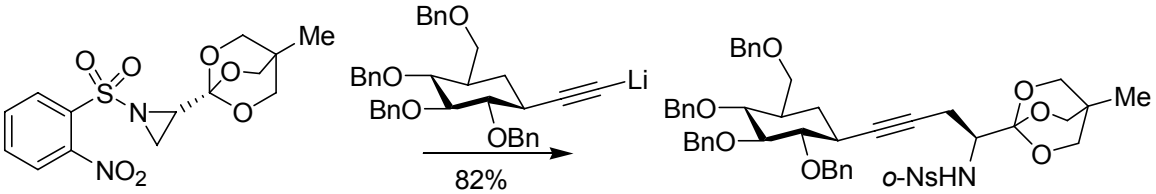


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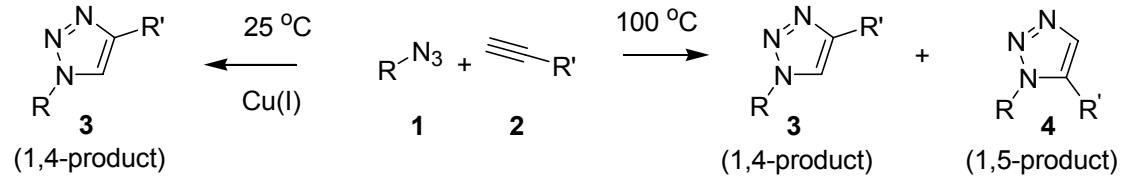
# 4 TOWARD TRIAZOLE-BASED CYSTINE MIMICS

## 4.1 Introduction

During the course of our investigations, new developments in acetylene chemistry culminated in 2002 in two independent contributions describing a catalytic system which enhances azide-alkyne 1,3-dipolar cycloadditions resulting in [1,2,3]-triazoles.




**Scheme 1**. *Huisgen cyclization leads to two regioisomers vs Cu(I)-catalyzed variant*

This so-called Huisgen reaction generally requires harsh conditions (refluxing in toluene) and produces a mixture of the two possible regioisomeric 1,4- (**3**) and 1,5-triazoles (**4**). The first contribution of the group of Meldal describes a screening of metal complexes that are able to catalyze this particular cycloaddition.[1,2] They found that copper(I) species gave selectively access to the 1,4-isomer in high yield. Furthermore, the application of this catalytic system to functionalize solid-phase bound peptides was described. They also state that the [1,2,3]-triazole moiety can be regarded as a peptide isostere that, when incorporated into a peptide, both displays hydrogen-bonding capabilities and mimics the amide backbone.

The second contribution from the Sharpless group described an identical reaction using a copper(I) catalyst.[3] They showed that an unprecedented level of selectivity and reliability was reached in this reaction, even in the creation of covalent links between densely functionalized building blocks. Interestingly, the conditions applied by the Sharpless group involve water as the primary solvent.

An added value of this reaction – currently commonly known as the 'click-reaction' – may lie in new generic applications for linking of different fragments under mild, so-called bio-orthogonal conditions.[4] Especially the azide combines

selective and high reactivity with stability in aqueous and/or oxygenated systems.

The copper(I)-mediated mechanism follows presumably the pathway outlined in Figure 1.[5] Recently, a thorough investigation has provided the insight that a dimeric copper species **8** may be responsible for the catalytic cycle. This may explain the greatly enhanced reactivity of a second azide in diazide-containing species.[6] A pre-organized catalyst thus eventually gives rise to the formation of ditriazoles over statistically expected mixtures, even when a 10-fold excess of diazide is used.

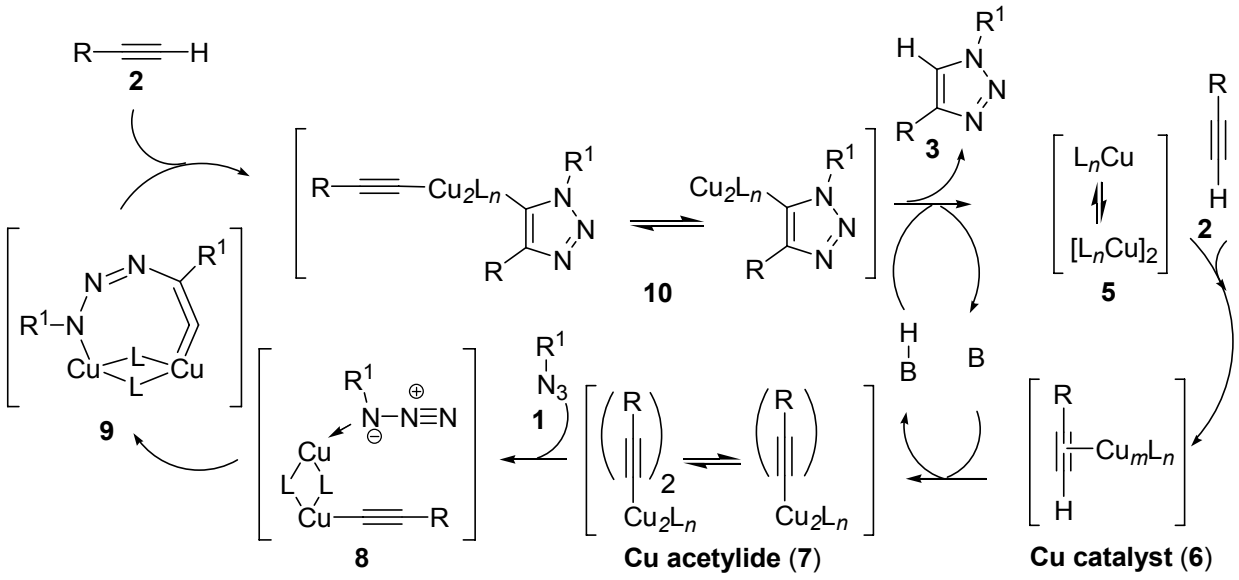
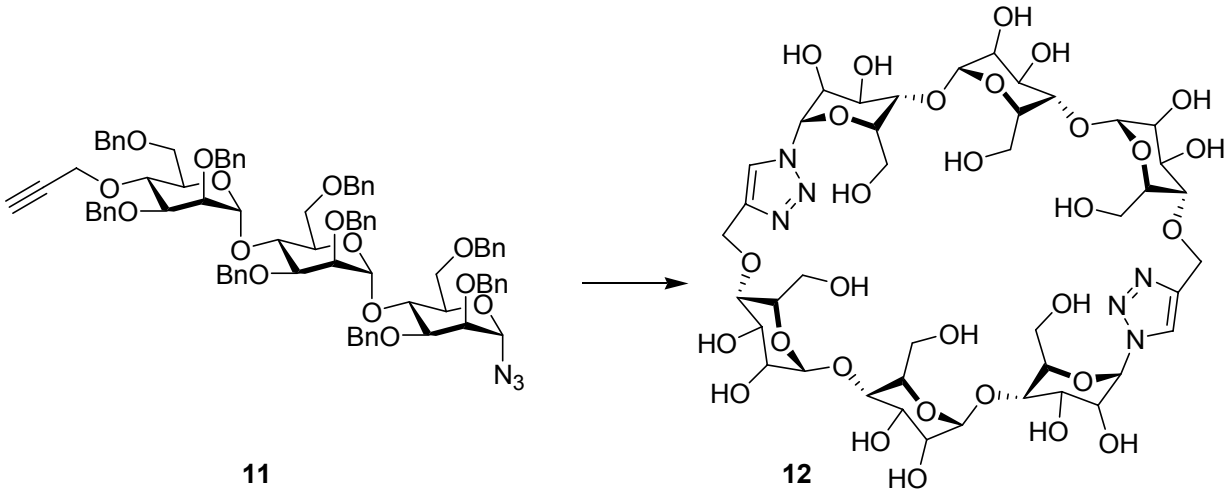



**Figure 1**. *Proposed mechanism of the click-reaction.*

First, the copper(I)-catalyst forms a π-complex with the acetylene (**6**) and subsequently replaces the acidic proton of the acetylene. The azide coordinates to the copper via the negatively charged nitrogen atom (**8**), followed by attack and subsequent formation of the new intermediate **9**. This slightly bent allene-like structure then rearranges to form the copper-triazole species **10**. Finally, copper(I) exchange with an acidic proton delivers the triazole (**3**) and the copper(I) species that can enter the next cycle.

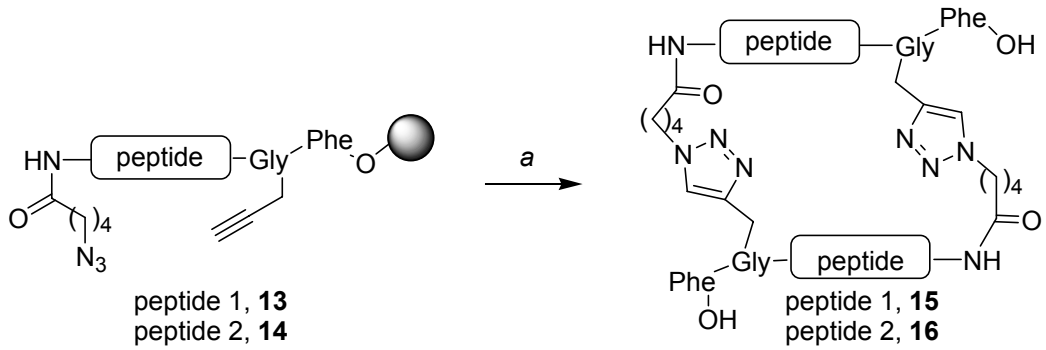
During the course of our investigation many examples of intramolecular cyclization appeared.[7] For example, Gin *et al.* used the copper-mediated 1,3-dipolar cycloaddition to obtain the dimeric cyclodextrin **12** in 80% yield (and 15% of its trimer). Later, even trimeric cyclodextrins[8] and $C_2$-symmetric cyclic peptides[9] were reported (Scheme 2).




**Scheme 2**. *Reagents and conditions: CuI, DBU, 50 °C then Pd/C, $NH_4HCO_2$ (80%, 2 steps).*

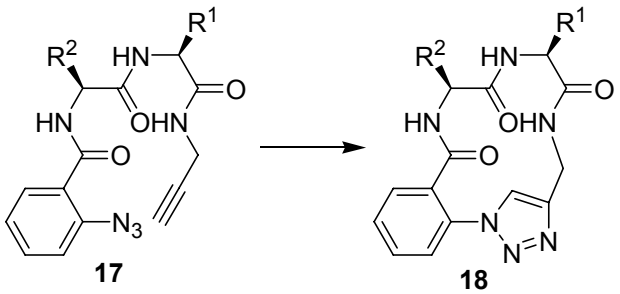
Head-to-tail peptide cyclizations were also investigated by Finn *et al*. (Scheme 3).[10] While long-term exposure of the immobilized azido-acetylene **13** or **14** to classic Huisgen cyclization conditions gave no reaction, Cu-catalysis provided the desired dimeric head-to-tail cyclization products (**15**, **16**)

.



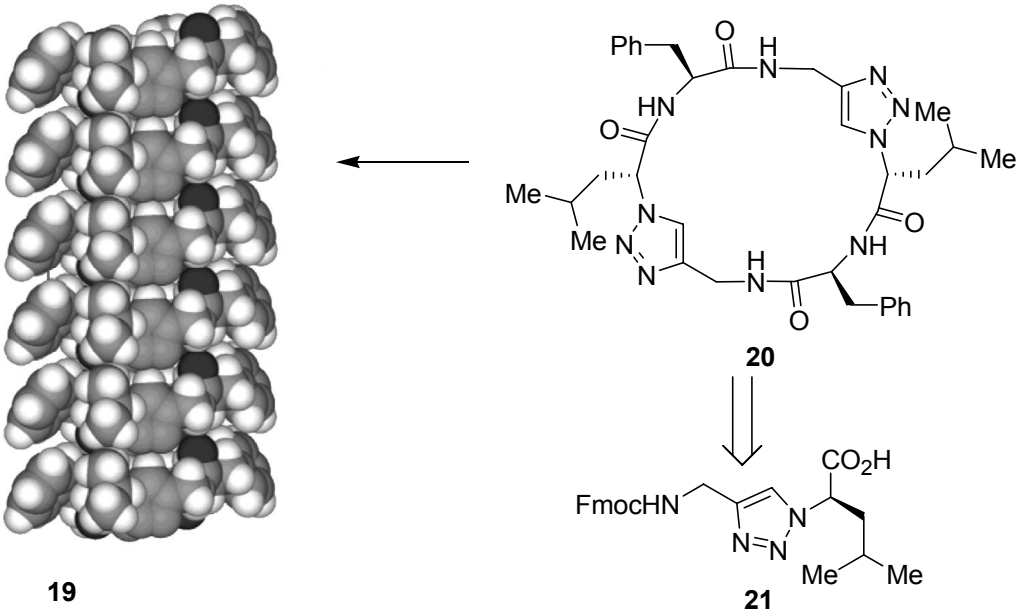

**Scheme 3**. *Reagents and conditions: a) i: CuI, ii: TFA. Peptide 1: Lys-Ala-Ile-Arg-Gly-Asp-Thr-Phe-Ala (**15**), 76 membered ring; Peptide 2: Lys-Met-Asn-Asp-Hys-Ala-Ile-Arg-Gly-Asp-Thr-Phe-Ala-Thr-Arg-Ala-Glu (**16**) 124 membered ring.*

Most recently, a ring closure was established in the Burgess group through an intramolecular click-reaction to form a ß-turn mimic (Scheme 4).[11] Remarkably, variation of the side chains $R^1$ and $R^2$ had a large influence on the monomer/dimer ratio, of 50% of the former, while the maximum isolated yield was 14%.




**Scheme 4**. *Reagents and conditions: Slow addition to CuI, DiPEA, THF, 25 °C, 14 h.*

Another example of 'intramolecular' applications by the Ghadiri group shows the compatibility of triazoles with peptide-containing systems.[12] They introduced triazole-containing dipeptides mimics **21** in cyclic octapeptides **20** which, analogous to the corresponding cyclopeptides,[13] formed an extended network resulting in the nanotubes **19** (scheme 5).




**Scheme 5**. *Nanotube **19** based on triazole-containing cyclopeptides **20**.*

Further studies by the Ghadiri group utilized dipeptide-mimics of types **21** in α-helical coiled coils to alter the secondary and quaternary structures for enhanced thermodynamic stability.[14] This example also proves that the triazole unit can be successfully applied in peptides as a conformational mimic of the amide bond.
Figure 2 displays our computational results for electron densities around the amide and triazole and illustrates the similarity between the two. The direction and also magnitude of the dipole moments are with 3.92 and 4.83 Debye respectively almost identical. This demonstrates the capability of the triazole to act as a hydrogen bond acceptor.

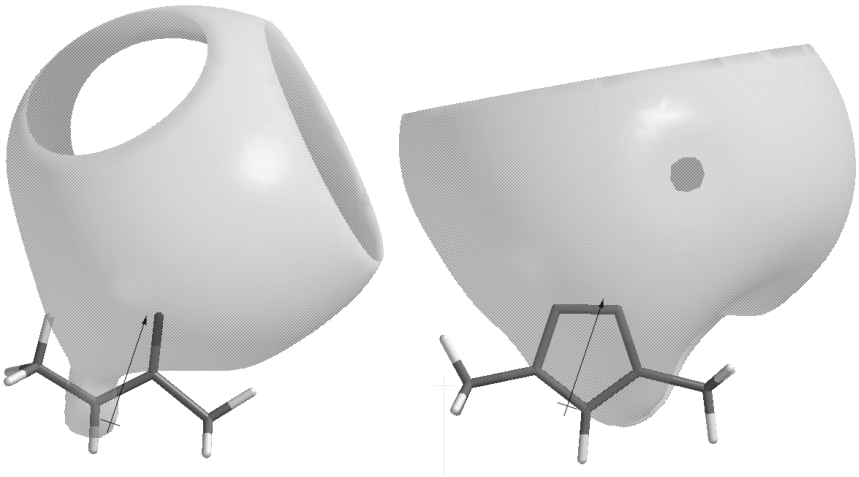
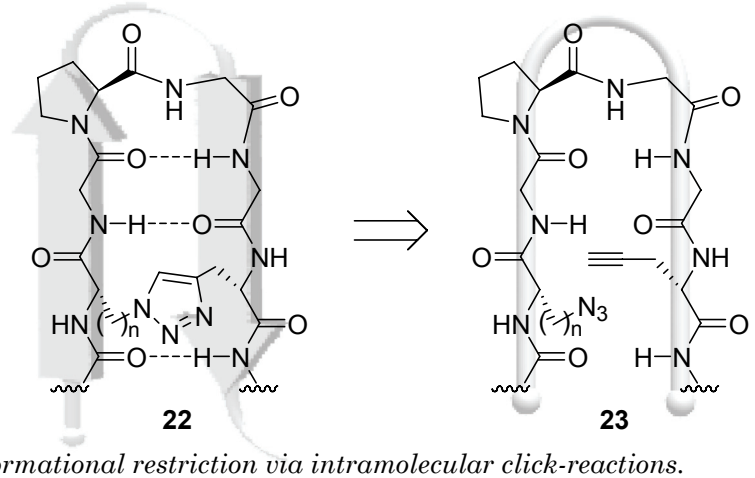


**Figure 2.** *Electron potential surfaces for an amide (left) and a triazole (right).*[15]

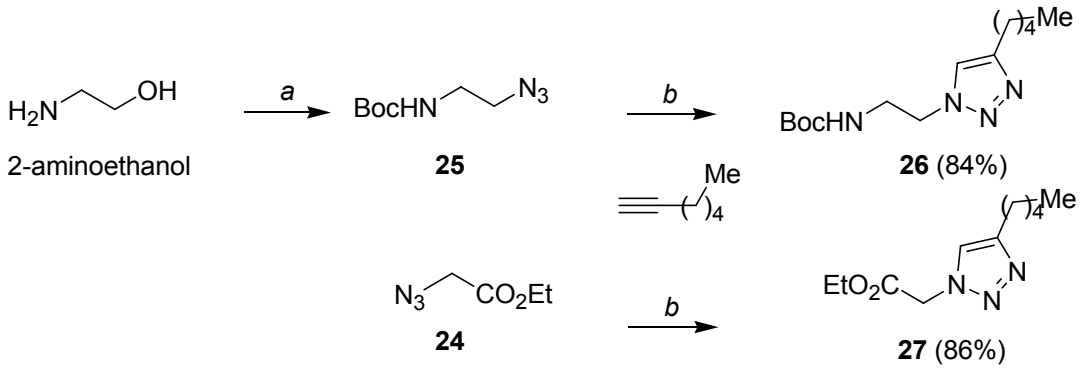
In this chapter, the use of azide/acetylene 1,3-dipolar cycloadditions to form conformationally restricted ß-turn mimics **22** is investigated (Scheme 6). The synthesis of suitable precursors **23** and the application of ring contractions are discussed.




**Scheme 6**. *Conformational restriction via intramolecular click-reactions.*

## *4.2 Intermolecular studies*

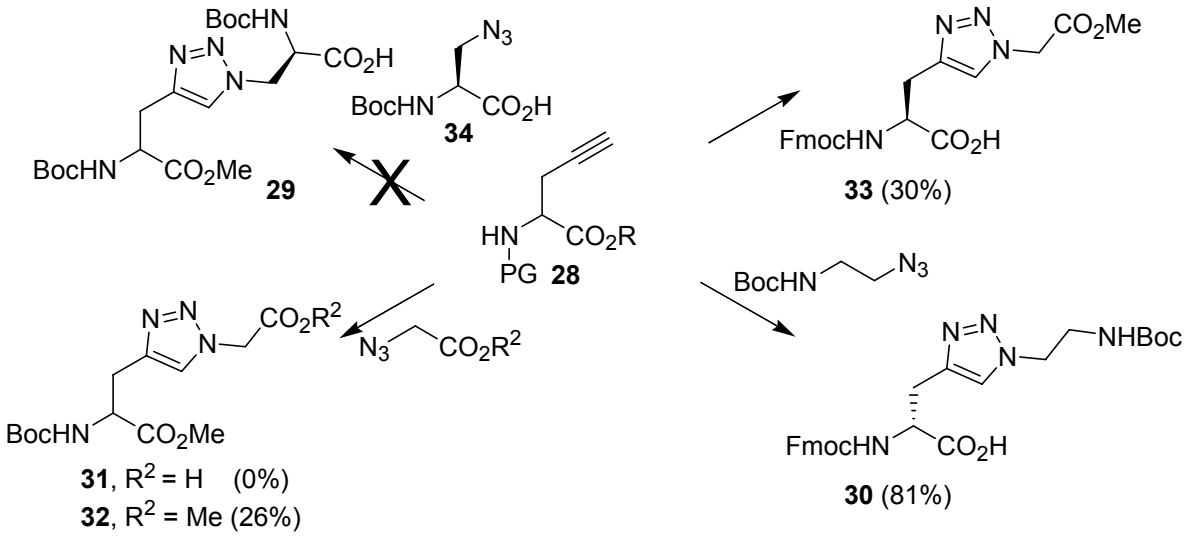
In order to test the click-reaction on amino acid-type structures, various azides were reacted with acetylenes using the Sharpless conditions (*in situ* reduction of Cu(II) with sodium ascorbate). The source of Cu(I) *via in situ* reduction is convenient, since copper(I) salts (CuI or CuOTf) are generally more difficult to handle. Furthermore, the formation of undesired byproducts such as Glaser type homocoupled acetylenes is suppressed under these reductive conditions.




**Scheme 7**. *Reagents and conditions: a) i: $Boc_2O$, $Et_3N$, THF, ii: MsCl, $CH_2Cl_2$, iii) $NaN_3$, DMF, 60 °C (54%, 3 steps); b: $Cu(OAc)_2$, Na-ascorbate, $H_2O$, $^tBuOH$ (2 : 1).*

Transformation of 2-aminoethanol to the desired precursor **25** was readily performed in reasonable yield via Boc-protection, mesylation and nucleophilic substitution using sodium azide. When the organic components (dissolved in $^t$BuOH) and the $Cu(OAc)_2$/sodium ascorbate couple (dissolved in water) were combined,[16] the solution turned bright yellow. After workup the organic phase contained the triazole **26** as a single product in good yield (84%). Glycine derived ethyl ester **24** was subjected to identical conditions, resulting in **27** as a single product.

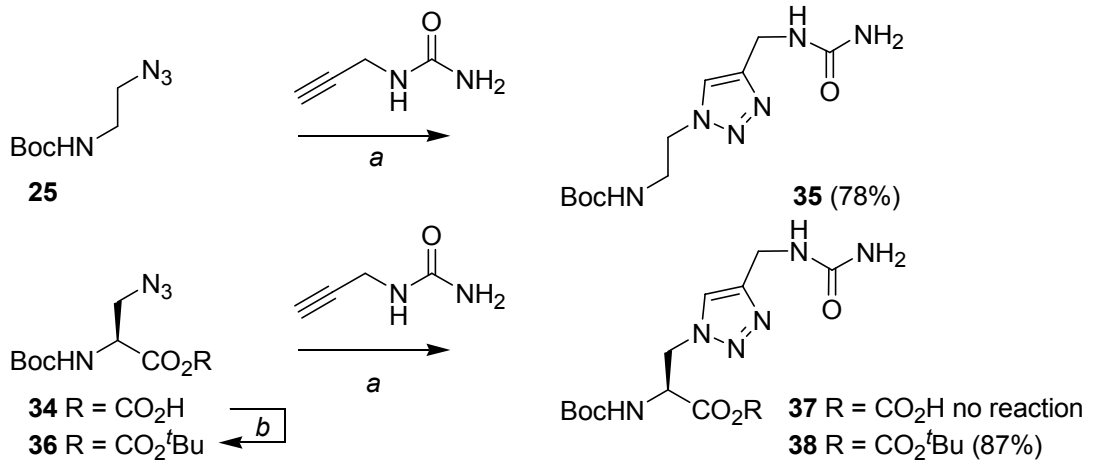
To further extend this methodology, several amino acids were combined in order to obtain isosteres that might be of use as cross-links in peptide synthesis (see also: Sections 3.5 and 5.1). Starting from suitably protected **28a** (PG = Boc, R = Me) of **28b** (PG = Fmoc, R = H), click reactions were carried out under the aforementioned conditions with different azides (Scheme 8). Remarkably, coupling of **28a** with Boc-protected azidoalanine was unsuccessful and also other partners gave variable results. According to $^1$H−NMR, compound **31** was formed, but could not be recovered from the water layer. As for the corresponding methyl ester protected product **32**; the product could be obtained in reasonable yield. 1,3-Dipolar cycloaddition of the Fmoc-protected propargylglycine **28b** to give **30** and **33** proceeded well. These preliminary results gave us the confidence that we should be able to apply such a strategy in a intramolecular fashion.




**Scheme 8**. *Reagents and conditions: $CuSO_4$, Na-ascorbate, $H_2O$, $^tBuOH$ (2 : 1).*

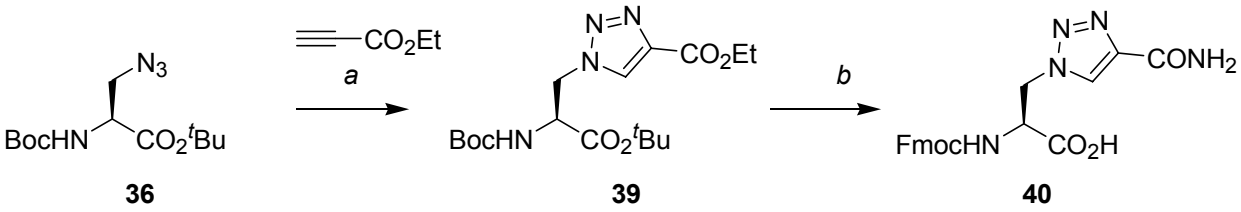
## *4.3 Citrulline derivatives*

As partly described in Section 3.4, rigidified glutamic acid or citrulline derivatives are biologically interesting building blocks. Similar derivatives may also be obtained by linking the amino acid moiety with the side chain functional group using the click-reaction, resulting in a rigid triazole spacer. An investigation combining azidoalanine **34** (obtained by diazotransfer on enantiopure 2,3-diaminopropanoic acid) with propargylurea gave,[17] however, no positive results when standard click-conditions were applied (Scheme 9). In contrast, the Boc-protected amino azide **25** gave 78% of the desired product. This led us to protect the free acid as a *tert*-butyl ester (**36**, obtained using the Kunz-conditions),[18] which under the same conditions gave rise to product **38** in 87% yield.




**Scheme 9**. *Reagents and conditions: a) $Cu(OAc)_2$, Na-ascorbate, $H_2O$, $^tBuOH$ (2 : 1); b) DCC, $^tBuOH$, CuCl, 3 days (88%).*

In comparison to citrulline, the side chain of urea derivative **38** is rather long. A shorter side chain in which the triazole may act as a hydrogen bridge acceptor was envisioned as shown in Scheme 10. Azide **36** was reacted with ethyl propiolate, after which the acid-labile protective groups were removed. Upon treatment with ammonia (25% aqueous solution) and subsequent Fmoc-protection, the desired amino acid **40** was obtained (44%, 4 steps). En passant, this example successfully demonstrated the possibility of conjugated acetylenes participating in the copper-catalyzed 1,3-dipolar cycloaddition.



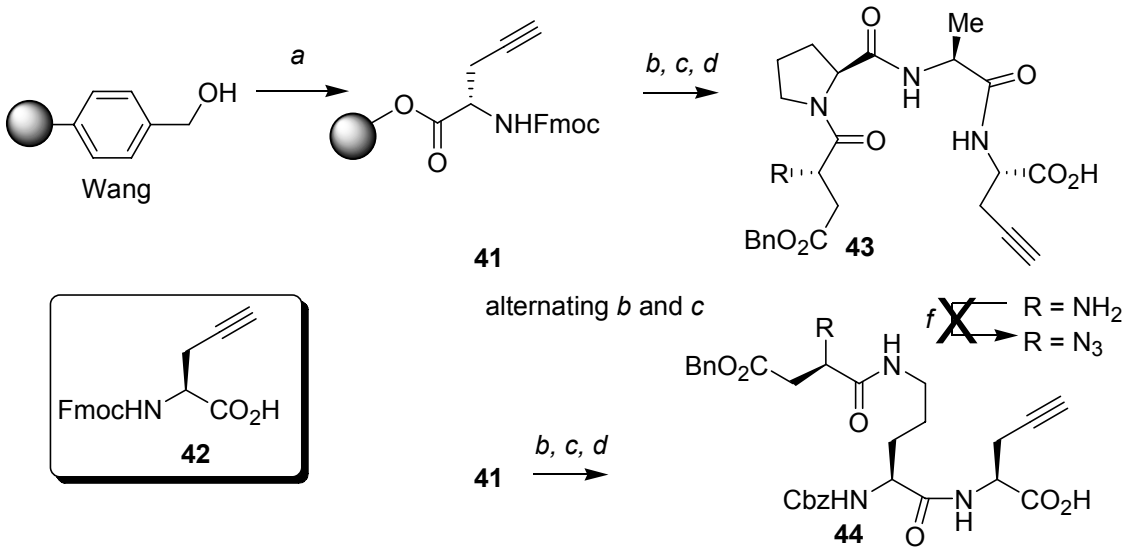

**36** **39** **40**

**Scheme 10**. *Reagents and conditions: a) Cu(OAc)₂, Na-ascorbate, H₂O, ᵗBuOH (2 : 1)(88%); b) i: TFA, ii: NH₄OH, iii) FmocOSu (51%, 3 steps).*

## *4.4 Peptide transformations*

Having established a proper protocol for intermolecular click-reactions on amino acid derivatives, our attention shifted to the corresponding intramolecular cycloadditions. Common approaches to introduce rigidification in peptides require non-polar solvents in which the peptide folding may be different from the folding in an aqueous environment. To the best of our knowledge, no examples of introduction of artificial peptide-stabilization in aqueous solvents are known.

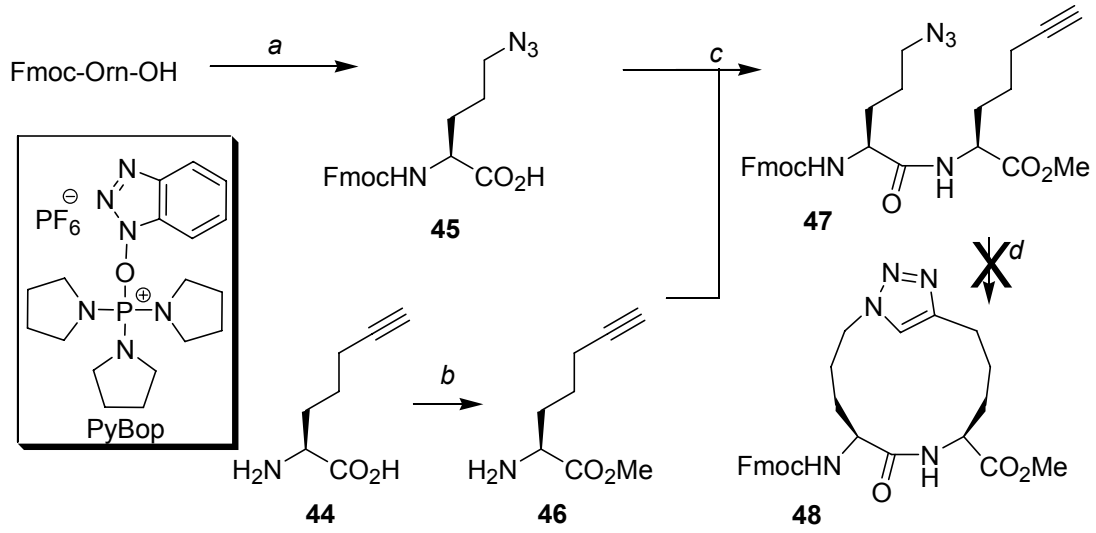
We then set out to investigate, whether the latter could be achieved with this click-reaction. Two oligopeptides, **43** and **44** (based on experiences as detailed in Section 5.4) were made using the required acetylene and azide handles. Both peptides **43** and **44** were constructed using standard solid phase reaction protocols. To a Wang-resin, Fmoc-protected propargylglycine (**42**) was coupled yielding **41** with a loading of 0.75 mmol/g. While in principle any amino acid would suffice, side-chain protected asparagine was chosen as the *N*-terminal amino acid in **43** and **44**. Alternating subsequent deprotection, standard peptide coupling and eventual cleavage of the unprotected *N*-terminus resulted in both **43** and **44** in good overall yields of 79 and 66%, respectively. Unfortunately, treatment of peptides **43** and **44** under the typical diazotransfer conditions with triflic azide furnished solely undefined products.[17]




**Scheme 11**. *Reagents and condtions: a) DIC, HOBt, DMAP, Fmoc-(S)-2-amino-4-pentynoic acid (**42**); b) DIC, HOBt, Fmoc-AA-OH, DMF; c) 0.1 M HOBt, 20% piperidine in DMF; d) 1% TFA in $CH_2Cl_2$; e) FmocOSu, $Et_3N$, MeCN, $H_2O$ (96%); f) $TfN_3$, $CuSO_4$, $CH_2Cl_2$, $H_2O$, MeOH.*

It was then suspected that the active copper(II)-catalyst for the azide-activation could also be involved in coordination to the acetylene, possibly leading to acetylene decomposition or undesired polymerization. Other model-systems such as **47** were initially made using Boc-protection and also these proved to be incompatible with triflic azide treatment.

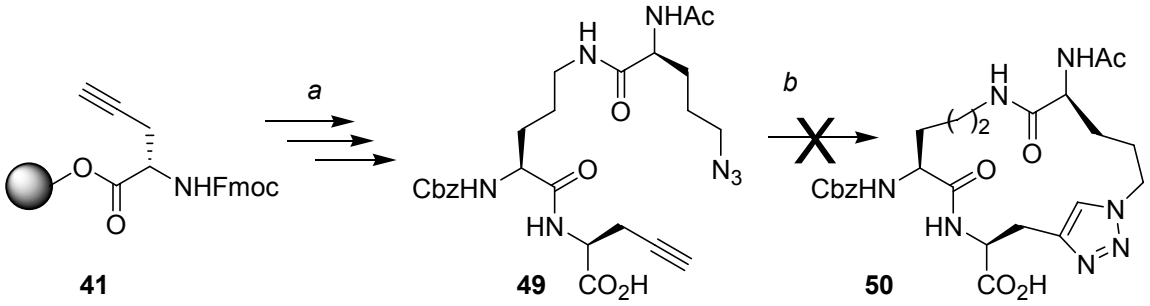
To still be able to probe the viability of such an approach, dipeptide **47**, a potential ß-turn mimic precursor as discussed in Sections 1.2 and 5.3, was prepared via coupling of (*S*)-2-amino-6-heptynoic acid methyl ester and Fmoc-Orn($N_3$)-OH with Castro's reagent in a moderate yield of 47% (Scheme 12).




**Scheme 12**. *Reagents and conditions: a) $TfN_3$, $CuSO_4$, $CH_2Cl_2$, $H_2O$, MeOH (95%); b) $SOCl_2$, MeOH, reflux (99%); c) PyBOP, DiPEA, $CH_2Cl_2$ (47%); d) $Cu(OAc)_2$, Na-ascorbate, $H_2O$, $^t$BuOH (2 : 1)*

Unfortunately, subsequent submission to copper(I) (*in situ* formed via reaction of copper(II) acetate with sodium ascorbate) did not result in a [2+3]-cycloaddition. In addition, subjection of **47** to copper(I) iodide in the presence of 2,6-lutidine at variable temperatures had no good results either. Presumably, the anticipated bicyclic system is too strained to be formed under these conditions so that alternative reaction pathways leading to polymerization may prevail.

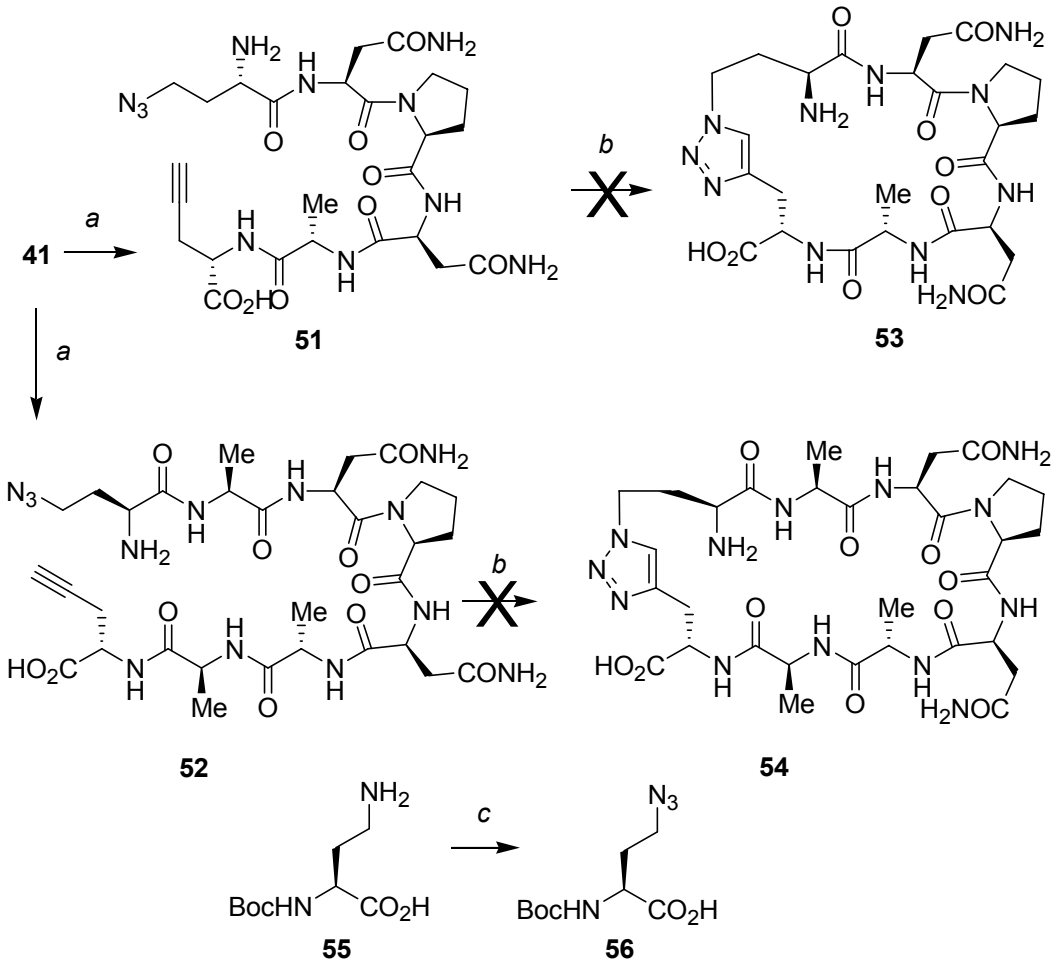
Despite these drawbacks, we intended to further pursue these types of cyclization reactions. In order to be able to faster generate peptide cyclization precursors, we changed to solid phase synthesis strategies involving acetylene- and azide-containing amino acids. This required a synthesis of the latter compounds, which was carried out starting from protected (*S*)-2-amino-4-pentynoic acid and azide functionalized ornithine, made using the aforementioned diazotransfer procedure. (Scheme 13). Several oligopeptides were thus synthesized and subjected to the copper-promoted 1,3-dipolar cycloaddition conditions.




**Scheme 13**. *Reagents and conditions: a) standard solid phase Fmoc-chemistry, followed by cleavage with TFA; b) Cu(OAc)₂, Na-ascorbate, H₂O, ᵗBuOH (2 : 1).*

The first example was tripeptide **49**,[19] the ornithine residue may act as a ß-turn inducer, allowing the acetylene and the azide to interact in the desired manner. Unfortunately, this cyclization did not provide any identifiable products.
In order to maximize the chance for the two ends to react with each other, next the robust ß-hairpin sequence based on Asn-Pro-Asn-Ala was chosen.[20] The asparagine can form an additional H-bridge over the ß-turn, resulting in a rather stable ß-hairpin that is also frequently encountered in nature. Hence, the fully deprotected peptides **53** and **54** could be examples of conformational fixation in an aqueous environment. Construction of a small oligopeptide with two terminal 1,3-dipolar cycloaddition components would provide the required flexibility necessary for cyclization.

Using the aforementioned Fmoc-solid phase synthesis protocols, both the 6-mer **51** and the 8-mer **52** were prepared, starting from the Fmoc-protected propargylglycine functionalized Wang-resin **41** (Scheme 14). The portionwise piperidine treatment for Fmoc-deprotection was performed in the presence of 0.1 M HOBt to suppress aspartimide formation.[21,22] Using standard DIC/HOBt activation the suitably protected amino acids were consecutively attached. The *N*-terminal residue was made via diazotransfer on 2-amino Boc protected 2,4-diaminobutyric acid (**55**) and **56** was subsequently used in the solid phase synthesis.[23] Upon cleavage from the resin the trityl and *N*-terminal Boc-group were removed. Due to the reactive nature of the thus created cations and the presence of the acetylene, additives such as $^{i}Pr_3SiH$ and 1,2-ethanedithiol were added to the cleavage cocktail. After the usual workup mass spectroscopy confirmed the presence of **51** and **52**, while IR and NMR-spectroscopy confirmed the presence of the azide and the acetylene, respectively.

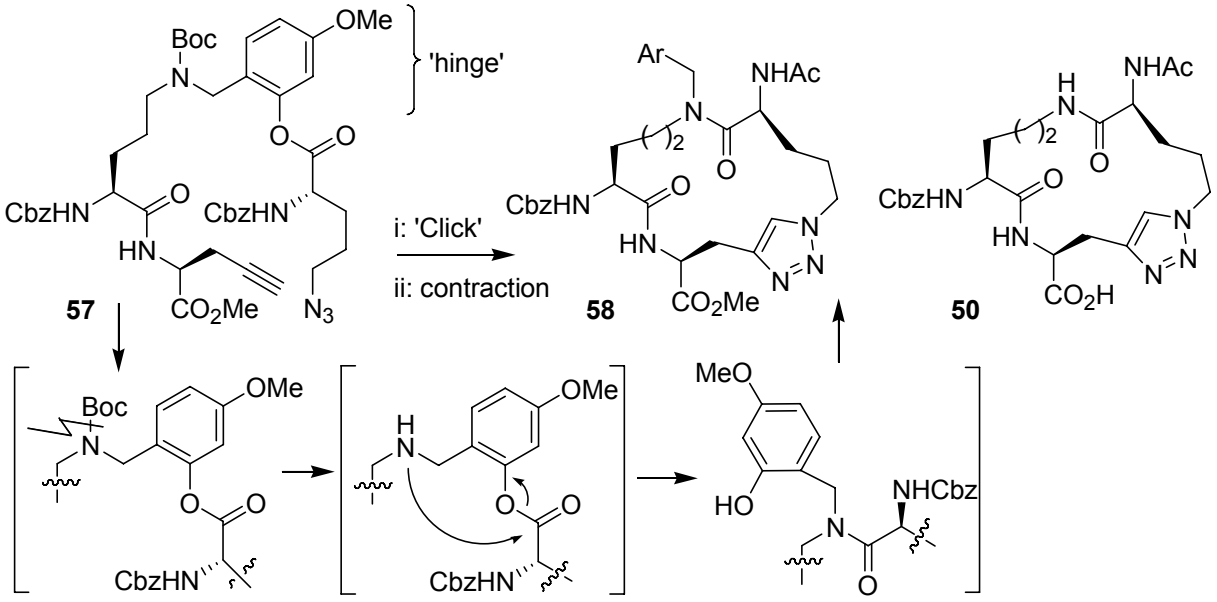



**Scheme 14**. *Reagents and conditions: a) standard solid phase (Fmoc) chemistry; b) $Cu(OAc)_2$, Na-ascorbate, $H_2O$, $^{t}BuOH$ (2 : 1) or 2,6-lutidine, DiPEA, CuI in deoxygenated MeCN; c) $TfN_3$, $CuSO_4$, $CH_2Cl_2$, $H_2O$, MeOH (95%).*

Initially, *in situ* reduced copper(II) in a 1 mM aqueous solution was used for the cyclization. Although some conversion took place in case of **52**, the resulting polar product was difficult to separate from residual copper salts, which is necessary for $^{1}$H−NMR analysis. Analytical HPLC showed that the starting peptide had been consumed, but a clear product peak could not be observed. Probably, either decomposition or polymerization might have taken place under these reaction conditions. Utilization of microwave irradiation solely resulted in decomposed products. An alternative copper(I) source in the form of CuI did not result in any recoverable peptides for both **51** and **52**. Focusing on **52**, more classical conditions such as refluxing in degassed toluene in the presence of CuBr (up to 1 equiv) and DBU showed no conversion at all, as based on LCMS analysis.
As a possible explanation for these failures, it was reasoned that chelation by the peptide to the copper catalyst (as observed with **35** to **37**) could diminish the copper catalysis. Hence, oligopeptide **52** was pretreated with $Ac_2O$ to convert free, reactive amines into amides and subsequently exposed to CuI, but also this did not change the outcome of the reaction.

## *4.5 Contraction strategies*

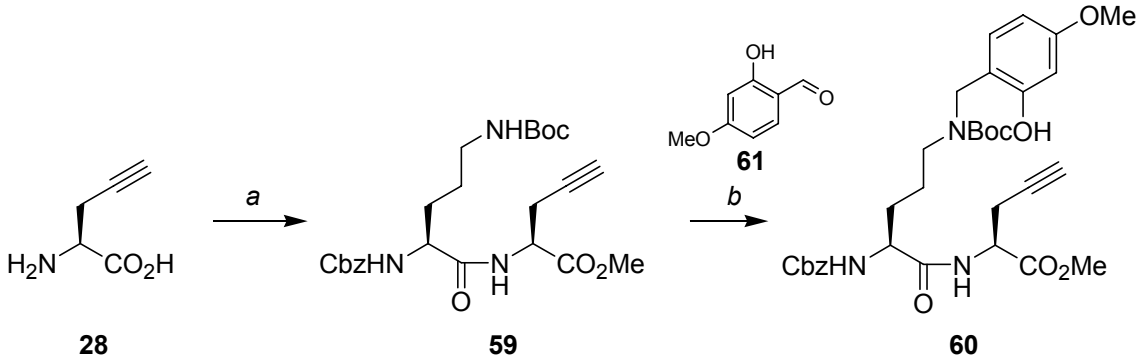
A novel method to prepare strained, cyclic peptides is currently being developed in the group of Hiemstra/van Maarseveen at the University of Amsterdam.[24] In this concept, a 'hinge' moiety is used which initially facilitates cyclization, and in a later stage can be activated for ring contraction (Scheme 15).




**Scheme 15**. *Concept of ring contraction.*

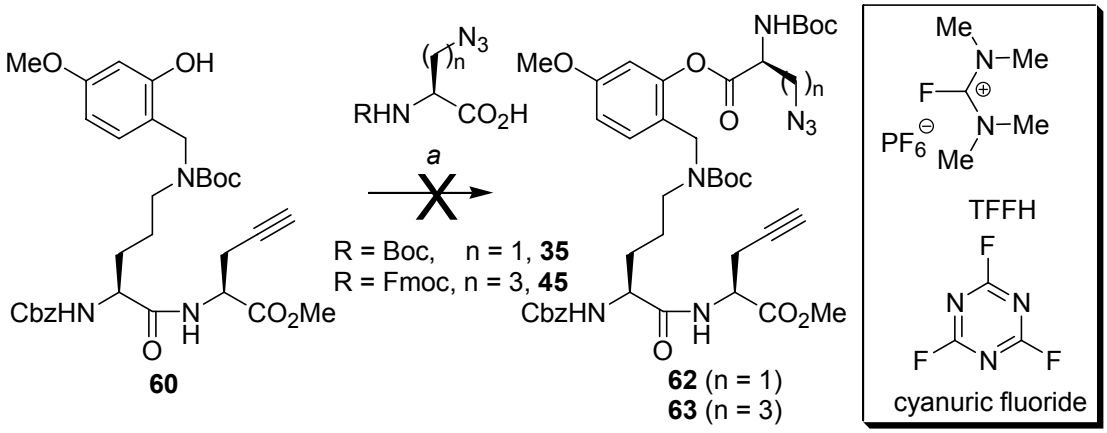
The phenolic moiety present in precursor **57** has been developed to serve those two purposes. After cyclization, Boc-deprotection triggers intramolecular amide bond formation, resulting in a ring contraction. The application of such a contraction might provide the targeted cyclic peptide systems **58**.

Since cyclization to **50** so far has been unsuccessful, we reasoned that oligopeptide **57** could be a nice example to demonstrate the viability of this methodology. The synthesis commenced with (*S*)-2-amino-4-pentynoic acid (**28**), which upon esterfication and subsequent coupling to Cbz-Orn(Boc)-OH, yielded dipeptide **59** (Scheme 16). After TFA-mediated removal of the Boc-group, the resulting amine was condensed with aldehyde **61** in the presence of 20 equiv of sodium sulfate as dehydration agent and the resulting imine reduced with excess sodium triacetoxyborohydride.[25] Subsequent Boc-protection using 1.2 equiv of $Boc_2O$ led to compound **60** in 23% overall yield.




**Scheme 16**. *Reagents and conditions: a) i: $SOCl_2$, MeOH; ii: PyBOP, DiPEA, Cbz-Orn(Boc)-OH (88%, 2 steps); b) i: TFA; ii: **40**, $Na_2SO_4$; iii: $NaB(OAc)_3H$; iv: $Boc_2O$ (23%, 4 steps).*

To reach the target molecule, esterfication between the phenolic moiety and an azido amino acid was required (Scheme 17). However, due to steric hindrance of the phenolic substituents, reagents such as DCC and DIC appeared ineffective in this esterfication. Fortunately, it was found that acyl fluorides were particularly useful to effect this transformation. Thus, Fmoc-Orn($N_3$)-OH (**45**) was transformed into the corresponding acid fluoride using either TFFH (tetramethylfluoroformamidinium hexafluorophosphate) or cyanuric fluoride.[26] An excess of the acid fluoride was then added to phenol **60**, but remarkably dipeptide **60** was recovered in a quantitative manner. Further attempts were abandoned due to lack of starting material and the complexity of the synthetic route.




**Scheme 17**. *Reagents and conditions: a) TFFH, $CH_2Cl_2$ or cyanuric fluoride, pyridine, $CH_2Cl_2$*

# *4.6 Conclusions*

In this chapter the application of copper catalyzed 1,3-dipolar cycloaddition is investigated. Several relative simple substrates are indeed easily modified using these conditions and thus a small library of amino acids was made, including mimics of citrulline. However, more advanced intramolecular applications were not successful. Small flexible tri- or tetrapeptides were not recovered after catalysis. The 6- and 8-mer sequences, which should by design adopt a suitable ß-turn conformation, failed to undergo a 1,3-dipolar cycloaddition. The use of a wide variety of reagents also did not change the outcome. This shows that such an approach is far from trivial, although more recently successful examples have emerged in literature.[9,11]

# *4.7 Acknowledgements*

We thank Dr. Jan van Maarseveen and Victoria Bock (University of Amsterdam) for their hospitality and assistance in the attempts to cyclize 8-mer **52**. Their contribution to Section 4.5 is also kindly acknowledged. Hans Adams (Radboud University Nijmegen) is acknowledged for his assistance and advice involving the solid phase peptide synthesis.

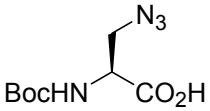
# *4.8 Experimental section*

For general experimental details, see: Section 2.8.

BocHN–N3 ***tert*-Butyl *N*-(2-azidoethyl)carbamate (25).**[27] To a solution of 2-amino-ethanol (1.50 g, 24.6 mmol) and $Boc_2O$ (5.90 g, 27.0 mmol) in 1,4-dioxane (60 mL) was added a solution of $NaHCO_3$ (4.13 g, 49.9 mmol) in $H_2O$ (60 mL). The

resulting mixture was refluxed for 2 h. After cooling down the organic solvents were stripped off using vacuum evaporation. The aqueous suspension was then extracted using $Et_2O$ and the combined organic layers were washed (brine), dried ($Na_2SO_4$) and evaporated. The resulting Boc-protected 2-aminoethanol was obtained as yellow oil (3.74 g, 23.3 mmol) which was directly used for the subsequent step. The oil was dissolved in $CH_2Cl_2$ (75 mL) and $Et_3N$ (3.9 mL, 26 mmol) was added. After cooling to 0 °C, MsCl (2.90 g, 25.4 mmol) was added dropwise and the resulting solution was stirred for 2 h at 0 °C. After warming to ambient temperature, $H_2O$ (50 mL) was added dropwise and the resulting aqueous layer was extracted using $CH_2Cl_2$ (2 × 50 mL). The combined organic layers were washed (brine), dried ($Na_2SO_4$) and evaporated. The resulting crude mesylated oil (6.40 g) was dissolved in DMF (75 mL) and sodium azide (3.24 g, 49 mmol) was added. The mixture was stirred at 60 °C overnight. After cooling down water (100 mL) was added and the mixture was extracted with heptane (3 × 75 mL). The combined organic layers were washed (brine), dried ($Na_2SO_4$) and evaporated, yielding the desired compound **25** (2.51 g, 13.6 mmol, 54%, 3 steps). $^1$H−NMR (400 MHz, $CDCl_3$) δ 4.81 (bs, 1H, NHBoc), 3.42 (t, $J$ = 5.1 Hz, $OCH_2$), 2H), 3.30 (q, $J$ = 5.4 Hz, 2H, $NCH_2$), 1.46 (s, 9H, Boc).

**3-Azido-(*S*)-2-*tert*-butoxycarbonylamino-propionic acid (34).**




A solution of sodium azide (6.36 g, 97.8 mmol) in distilled $H_2O$ (20 mL) and $CH_2Cl_2$ (20 mL) was cooled on an ice bath. Triflyl anhydride (5.52 g, 19.6 mmol) was added slowly over 5 min while stirring continued for 2 h. The mixture was placed in a separatory funnel and the $CH_2Cl_2$ phase removed. The aqueous portion was extracted with $CH_2Cl_2$ (2 × 10 mL). The organic fractions, containing the triflyl azide, were pooled and washed once with aqueous saturated $Na_2CO_3$ and used without further purification.

The amino acid Boc-DAP-OH (2.00 g, 9.80 mmol) was combined with $K_2CO_3$ (2.00 g, 14.8 mmol), $Cu(OAc)_2$ (catalytic amount), distilled $H_2O$ (20 mL) and MeOH (30 mL). The previously synthesized triflyl azide in $CH_2Cl_2$ (40 mL) was added, and the mixture was stirred at ambient temperature overnight. Subsequently, the organic solvents were removed under reduced pressure, and the aqueous slurry was diluted with $H_2O$ (50 mL). This was acidified to pH = 6 with concentrated HCl and extracted with EtOAc (2 × 30 mL) for removal of the sulfonamide byproduct. The aqueous phase was then acidified to pH = 2 with concentrated HCl. The product was obtained from EtOAc extractions (3 × 30 mL). The organic extracts were combined, dried ($MgSO_4$), and evaporated to dryness giving **34** as a

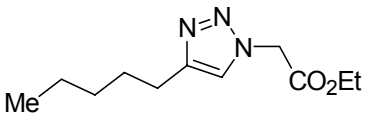
pale oil (1.92 g, 8.34 mmol, 85%) with no need for further purification. $^1$H−NMR (500 MHz, DMSO-$D_6$) δ 6.89 (bd, 1H, NH), 4.13 (ddd, *J* = 6.3 Hz, 6.3 Hz, 7.4 Hz, 1H, CαH), 3.37 (m, 2H, $CH_2N_3$), 1.39 (s, 9H, Boc); $^{13}$C−NMR (125 MHz, DMSO-$D_6$ δ 170.6, 154.8, 53.3, 50.8, 27.8; IR v 3329 br, 2980, 2106 str, 1714 cm$^{-1}$; HRMS m/e calcd for $C_8H_{18}N_5O_4$ + (M + $NH_4$)$^+$: 248.1359, found: 248.1356.

**3-Azido-(*S*)-2-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (36).**[27] A mixture of DCC (2.84 g, 13.0 mmol), $^t$BuOH (1.30 g, 17.6 mmol) and copper-(I)-chloride (15 mg, 0.15 mmol) was stirred for 3 days. The green suspension was then diluted with dry $CH_2Cl_2$ (5 mL) and the Boc-Ala($N_3$)-OH (**34**, 1.00 g, 4.34 mmol) in $CH_2Cl_2$ (5 mL) was added dropwise. The reaction was finished in 3 h as monitored by TLC. Precipitated urea was then removed by filtration. The organic layer was washed using saturated. $NaHCO_3$ solution (3 × 10 ml), dried ($MgSO_4$) and concentrated *in vacuo*, yielding *tert*-butyl ester protected amino acid **36** (1.10 gram, 3.84 mmol, 88%). $^1$H−NMR[28] (400 MHz, $CDCl_3$) δ 5.35 (bd, *J* = 6.6 Hz, 1H, NH), 4.36−4.31 (m, 1H, CαH), 3.73−3.63 (m, 2H, $CH_2N_3$), 1.49 (s, 9H, Boc), 1.46 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 83.1, 80.7, 55.0, 52.8, 27.3, 27.5.

**Fmoc-Orn($N_3$)-OH (45).**[27] Following the general procedure for diazotransfer as described above, Fmoc-Orn-OH (1.00 gram, 2.55 mmol) was converted to its corresponding azide **45** (924 mg, 2.41 mmol, 95%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.59−7.53 (m, 2H, Fmoc), 7.39 (t, *J* = 7.3 Hz, 2H, Fmoc), 7.33−7.26 (m, 2H, Fmoc), 5.40 (bd, *J* = 6.8 Hz, 1H, NH), 4.46−4.31 (m, 1H, CαH), 4.20 (t, *J* = 6.4 Hz, 1H, CαH-Fmoc), 4.12 (q, *J* = 7.3 Hz, 2H, $CH_2O$), 3.35−3.26 (m, 2H, $CH_2N_3$), 2.01−1.54 (m, 4H, 2 × $CH_2$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 173.3, 155.9, 143.6, 140.6, 140.5, 127.5, 127.4, 126.9, 125.1, 120.0, 119.9, 65.7, 53.6, 50.4, 46.9, 46.8, 28.3, 25.3; IR v 3383, 3317, 3065, 2951, 2098, 1705, 1525, 1450, 1393, 1235, 1195 cm$^{-1}$.

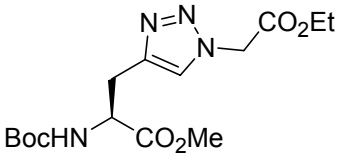
***tert*-Butyl *N*-[2-(4-pentyl-1*H*-1,2,3-triazol-1-yl)ethyl]carbamate (26).** To a stirred solution of *tert*-butyl *N*-(2-azidoethyl)carbamate (150 mg, 0.92 mmol) and heptyne (266 mg, 2.77 mmol) in $^t$BuOH (5 mL) was added a premixed solution of $Cu(OAc)_2$ (36 mg, 0.18 mmol) and sodium ascorbate (73 mg, 0.37 mmol) in $H_2O$ (5 mL). After vigorously stirring overnight, the

mixture diluted with $H_2O$ (10 mL) and extracted with $CHCl_3$ (3 × 7 mL), yielding **26** as a yellowish solid (217 mg, 0.77 mmol, 84%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 1H, ArH), 4.87 (s, 1H, NHBoc), 4.43 (t, *J* = 5.8 Hz, 2H, $NCH_2$), 3.62 (q, *J* = 5.8 Hz, 2H, $ArCH_2$), 2.71 (t, *J* = 7.6 Hz, 2H, NHC*H*$_2$), 1.71−1.31 (m, 6H, 3 × $CH_2$), 1.43 (s, 9H, Boc), 0.90 (t, *J* = 7.1 Hz, 3H, $CH_2$*Me*); $^{13}$NMR (75.5 MHz, $CDCl_3$) δ 155.2, 147.9, 120.8, 79.4, 49.4, 40.2, 31.2, 28.9, 28.1, 25.4, 22.2, 13.8; IR ν 3255, 2953, 2929, 2869, 1701, 1544, 1454, 1365, 1175, 972 cm$^{-1}$; HRMS (EI) m/z calcd for $C_{14}H_{26}N_4O_2$ 282.2056, found 282.20607.

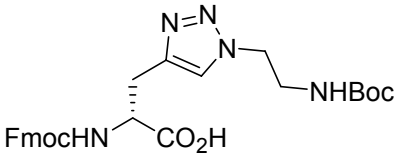



**Ethyl 2-(4-pentyl-1*H*-1,2,3-triazol-1-yl)acetate (27).** Following a general protocol as shown for **26**, $N_3$-Gly-OEt (130 mg, 1.00 mmol) and heptyne (192 mg, 2.00 mmol) were combined. After workup, the addition of hexane caused the desired product **27** (193 mg, 0.86 mmol, 86%) to crystallize as white needles. $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H, ArH), 5.15 (s, 2H, $ArCH_2CO$), 4.28 (q, *J* = 7.1 Hz, 2H, $OCH_2$), 2.75 (t, *J* = 7.6 Hz, 2H, $ArCH_2$), 1.71−1.63 (m, 2H, C*H*$_2$$CH_2Ar$), 1.40−1.30 (m, 4H, 2 × $CH_2$), 1.26 (t, *J* = 7.1 Hz, 3H, Me), 0.89 (t, *J* = 7.1 Hz, 3H); $^{13}$NMR (75.5 MHz, $CDCl_3$) δ 165.8, 148.3, 121.3, 62.0, 50.5, 31.2, 28.8, 25.4, 22.2, 13.9, 13.8; IR ν 2929, 2860, 2095, 1751, 1216, 773 cm$^{-1}$; MS (EI) m/z calcd for $C_{11}H_{19}N_3O_2$ 225.1477, found 226.1547 $(M + H)^+$.

**(2*S*)-methyl 2-[(*tert*-butoxycarbonyl)amino]-3-[1-(2-ethoxy-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl]propanoate (32).** Following a general protocol as shown for **26**, (S)-2-*tert*-butoxycarbonylamino-4-pentynoic acid methyl ester (**28**, 100 mg, 0.44 mmol) and $N_3$-Gly-OEt (57 mg, 0.44 mmol) were reacted. After isolation, **32** was yielded as a yellowish solid (40 mg, 0.112 mmol, 26%). $^1$H−NMR (400 MHz, $CDCl_3$) 7.52 (s, 1H, ArH), 5.51 (bd, *J* = 16.4 Hz, 1H, NHBoc), 5.13 (s, 2H, $CH_2N$), 4.70−4.57 (m, 1H, CαH), 4.26 (q, *J* = 14 hz, 2H, $CH_2Ar$), 3.73 (s, 3H, OMe), 3.27 (d, *J* = 10.8 Hz, 2H, $OCH_2$), 1.43 (s, 9H, Boc), 1.30 (t, *J* = 14 Hz, 3H, $CH_3$); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 171.5, 165.8, 155.0, 143.0, 123.2, 80.0, 62.4, 53.1, 52.5, 50.9, 28.4, 14.3; IR ν 3384, 2979, 1747, 1711, 1509, 1214, 1165 cm$^{-1}$.

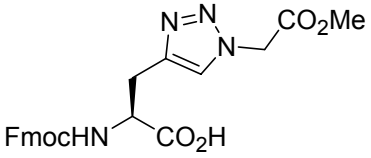


**(2*R*)-3-(1-2-[(*tert*-butoxycarbonyl)amino]ethyl-1*H*-1,2,3-triazol-4-yl)-2-[(9*H*-9-fluorenylmethoxy)carbonyl]aminopropanoic acid (30).**



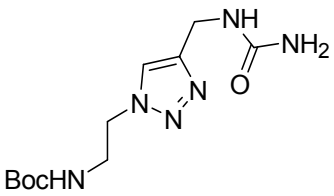

To a stirred solution of (*R*)-2-amino-4-pentynoic acid (400 mg, 3.54 mmol) in $H_2O$ (4 mL) were added $Et_3N$ (0.50 mL, 3.56 mmol) and FmocOSu (1.19 g, 3.53 mmol) which was dissolved in MeCN (10 mL). The pH of this mixture was maintained pH = 9.0 for 10 minutes by manual addition of $Et_3N$. After 15 more minutes $N_3$-Et-NHBoc (**25**, 580 mg, 3.54 mmol), $Cu(OAc)_2 \cdot 2H_2O$ (18.0 mg, 0.044 mmol) and Na-ascorbate (35.0 mg, 0.088 mmol, 0.4 equiv) were added. After 10 seconds, the suspension colored bright yellow. The mixture was stirred overnight. The pH was lowered to 6 and the organic solvents were stripped *in vacuo*. After further lowering the pH (< 2), extraction using EtOAc (3 × 10 mL), subsequent washing of the combined organic layers (brine, 15 mL), drying ($MgSO_4$) and evaporation, a brown solid was yielded. Flash chromatography (5% MeOH and 2% AcOH in $CH_2Cl_2$) resulted in **30** in off-white crystals (1.372 g, 2.63 mmol, 81%, 2 steps). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.8 Hz, 2H, Fmoc), 7.59 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.42−7.35 (m, 3H, Fmoc + triazole-H), 7.31 (dt, *J* = 1.0 Hz, 7.8 Hz, 2H, FMoc), 6.00 (bs, 1H, NH), 4.95 (bs, 1H, NHBoc), 4.70−4.62 (m, 1H, CαH), 4.43−4.32 (m, 4H, $CH_2O$, $CH_2NAr$), 4.22 (t, *J* = 6.4 Hz, 1H, Fmoc-αH), 3.60−3.55 (m, 2H, C*H*$_2$NHBoc), 3.40−3.30 (m, 2H, $CH_2CAr$), 1.42 (s, 9H, Boc); $^{13}$NMR (75.5 MHz, $CD_3OD$) δ 176.3, 156.3, 156.1, 143.2, 143.1, 140.5, 126.8, 126.2, 124.3, 124.3, 78.7, 66.3, 49.4, 39.8, 27.3, 27.1; IR ν 2985.7, 2899.7, 1715.5, 1406.0, 1227.2, 1054.7 $cm^{-1}$.

**(2*S*)-3-[1-(carboxymethyl)-1*H*-1,2,3-triazol-4-yl]-2-[(9-fluorenylmethoxy)carbonyl]aminopropanoic acid (33).**

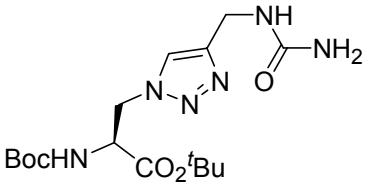



Using the previously described protocol, (*S*)-2-amino-4-pentynoic acid (500 mg, 4.42 mmol) was protected subsequently combined with $N_3$-Gly-OMe (508 mg, 4.42 mmol), yielding **33** as off-white crystals (720 mg, 1.60 mmol, 38%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.8 Hz, 2H, Fmoc), 7.59 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.42−7.35 (m, 3H, Fmoc + ArH), 7.31 (dt, *J* = 1.0 Hz, 7.8 Hz, 2H, Fmoc), 5.87 (bd, *J* = 6.8 Hz, NH), 5.14 (t, *J* = 21.0 Hz, 2H, Ar-$CH_2CO_2Me$), 4.68−4.63 (m, 1H, CαH), 4.51−4.36 (m, 2H, $CH_2O$), 4.22 (t, *J* = 6.4 Hz, 1H, Fmoc-αH), 3.78 (s, 3H, OMe), 3.47−3.32 (m, 2H, $CH_2Ar$); $^{13}$NMR (75.5 MHz, $CD_3OD$) δ 176.2, 170.6, 160.3, 147.2, 147.1, 144.4, 130.7, 130.1, 128.3,

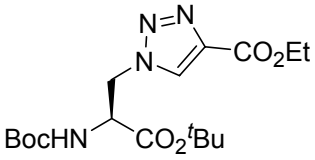
127.8, 122.9, 70.3, 57.3, 55.5, 53.8, 51.0, 31.1; HRMS (EI) m/z calcd for $C_{23}H_{22}N_4O_6$ 450.15393, found 450.15604.

***tert*-Butyl *N*-[2-(4-[(aminocarbonyl)amino]methyl-1*H*-1,2,3-triazol-1-yl)ethyl]carbamate (34).** 

Using the general procedure, *tert*-butyl 2-azidoethylcarbamate (200 mg, 1.26 mmol) was combined with 1-(prop-2-ynyl)urea (123 mg, 1.26 mmol). Further purification (5% MeOH in $CH_2Cl_2$) yielded the desired urea **34** (251 mg, 0.98 mmol 78%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H, C=CH), 6.24 (s, 1H, N*H*CONH$_2$), 5.42 (s, 1H, NHBoc), 4.91 (m, 2H, $CH_2$NAr), 4.45 (t, *J* 4.9 Hz, 2H, C*H*$_2$NHCONH$_2$), 4.42−4.35 (m, 2H, CONH$_2$), 3.60 (q, *J* = 5.4 Hz, 2H, C*H*$_2$NHBoc), 1.43 (s, 9H, Boc); $^{13}$NMR (75.5 MHz, $CD_3OD$) δ 163.8, 160.1, 126.4, 82.6, 63.8, 53.3, 43.8, 38.7, 23.3, 17.7.

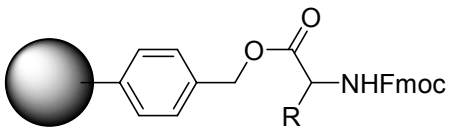
**[1-(*tert*-butoxycarbonyl)amino-2-(5-ureidomethyl-[1,2,3]triazol-1-yl)-ethyl]-carbamic acid *tert*-butyl ester (38).** 

Using the general procedure Boc-Ala($N_3$)-O$^t$Bu (**36**, 200 mg, 0.70 mmol) was combined with 1-(prop-2-ynyl)urea (68 mg, 0.69 mmol). Further purification (5% MeOH in $CH_2Cl_2$) yielded the desired urea **38** (235 mg, 0.61 mmol) in 87%. $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H, ArH), 6.30 (bs, 1H, N*H*CONH$_2$), 5.57 (bs, 1H, NHBoc), 4.92−4.85 (m, 2H, $CH_2$NAr), 4.77−4.70 (m, 2H, NH$_2$), 4.59−4.53 (m, 1H, CαH), 4.44−4.40 (m, 2H, ArC$H_2$N), 1.44 (s, 9H, Boc), 1.43 (s, 9H, Boc); $^{13}$NMR (75.5 MHz, $CDCl_3$) δ 167.7, 158.9, 154.9, 145.5, 12c3.2, 83.6, 80.5, 54.3, 51.1, 50.6, 35.4, 28.4, 28.0; IR ν 3348, 2978, 2934, 1708, 1657, 1367, 1152, 1053, 732 cm$^{-1}$; HRMS (EI) m/z calcd for $C_{16}H_{29}N_5O_5$ 385.2199, found 385.21968.

**Ethyl 1-3-(*tert*-butoxy)-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl-1*H*-1,2,3-triazole-4-carboxylate (39).** 

Using the general procedure Boc-Ala($N_3$)-O$^t$Bu (1.08 g, 3.94 mmol) was coupled to propynoic acid ethyl ester (580 mg, 5.91 mmol), resulting in triazole **39** (1.33 g, 3.45 mmol, 88%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H, C=CH), 5.46 (d, *J* = 6.8 Hz, 1H, NH), 4.79−4.76 (m, 2H, OC$H_2$), 4.52−4.43 (m, 1H, CαH), 4.36−4.28 (m, 2H, NC$H_2$), 1.39 (s, 9H, Boc), 1.36 (s, 9H, Boc), 1.32 (t, *J* = 7.0 Hz, 3H, $CH_3$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 167.0, 160.0, 154.6, 139.5, 128.3, 83.6, 80.3, 61.0, 53.9, 50.1, 28.0, 27.7; IR ν 3358.3, 2977.9, 2933.7, 2358.8, 1717.9, 1511.1, 1152.6, 1041.1 cm$^{-1}$.

*General procedure for the loading of Wang-resin.*



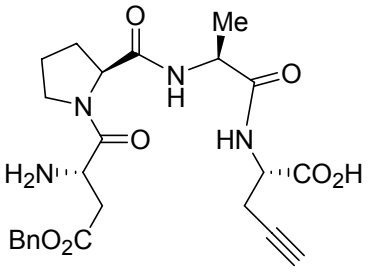

A batch of resin was allowed to swell in dry DMF. After removal of the DMF, fresh dry DMF was added, followed by the amino acid (2 equiv), DIC (2 equiv), HOBt (4 equiv) and DMAP (2 equiv). After 16 h the solution was removed and washed thrice with DMF. Then $Ac_2O$ (10% in DMF) was added in order to cap all remaining free sites. After removal of the solution the resin was washed twice using MeOH, twice with $CH_2Cl_2$ and twice with $Et_2O$ in order to remove all traces of DMF and dried. The loading was determined by measuring weight or Fmoc-titration.

*General procedure for amino acid coupling using Fmoc-NH-Resin.*

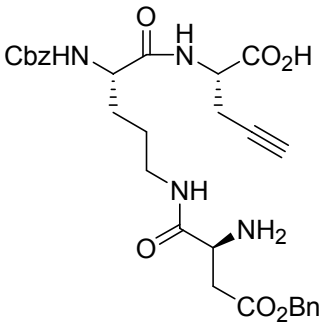
The Fmoc-protected amino acid loaded resin was treated with 20% piperidine and 0.1 M HOBt in DMF (15 mL) for 5 min and subsequently the solvent was removed (by filtration). This procedure was repeated three times. The resin was washed using DMF (3 × 1 min). Having removed the Fmoc, the resin was treated with DIC (3.3 equiv, 1 M in DMF), HOBt (3.3 equiv, 1 M in DMF), the Fmoc-AA-OH (3 equiv) and DMF and 'stirred' for approximately 40 min . The coupling was qualitatively monitored using the Kaiser test.[29] When completed, the solvent was again removed by filtration and washed with DMF (3 × 1 min).

*General procedure for the cleavage from the Wang-resin.*

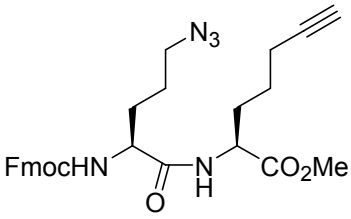
The resin was washed twice using MeOH, twice with $CH_2Cl_2$ and twice with $Et_2O$ in order to remove all traces of DMF. Then 1% of ethanedithiol, 1% of $H_2O$ and 1% of $^{i}Pr_3SiH$ in TFA were added and the resin was mixed for 4 h. After filtration, the filtrate was concentrated, dissolved in MeOH and treated with $Et_2O$, causing the peptide-chain to precipitate from the solution. The solid peptide was dissolved in dioxane and freeze-dried under vacuum, yielding a white solid.



**$H_2N$-Asp(Bn)-Pro-Ala-Prg[30]-OH (43).** Using standard solid phase chemistry, the Wang-resin (0.832 g, 0.75 mmol/g) was functionalized. After cleavage with TFA (50% in $CH_2Cl_2$) and evaporation under a stream of $N_2$, the solid was taken up in dioxane and freeze-dried, yielding **43** as a white solid (240 mg, 0.49 mmol, 79%). $^1$H–NMR (400 MHz, $CD_3OD$) δ 7.31 (s, 5H, Ar), 5.10 (s, 2H, $CH_2O$), 4.57–4.50 (m, 1H, CαH), 4.47–4.40 (m, 1H, CαH), 4.37–4.33 (m, 1H, CαH), 4.23–4.14 (m, 1H, CαH), 3.67–3.54 (m, 1H, $CH_2N$), 3.50–3.41 (m, 1H, $CH_2N$), 3.10–2.96 (m, 1H, C*H*$_2$CO$_2$Bn), 2.92–2.82 (m, 1H, C*H*$_2$CO$_2$Bn), 2.67–2.56 (m, 2H, C≡C$CH_2$), 2.27 (s, 1H, C≡CH), 2.26–2.15 (m, 1H, $CH_2$), 1.94–1.77 (m, 3H, $CH_2$), 1.27 (d, *J* = 6.8 Hz, 3H, Me); HRMS (EI) m/z calcd for $C_{24}H_{30}N_4O_7$ 486.21145, found 523.21987.

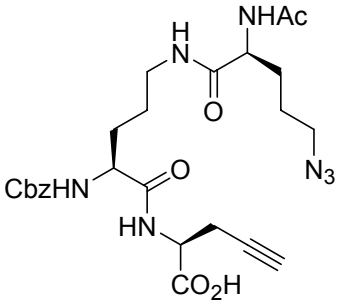


**Cbz-Orn(Asp(Bn))-Prg-OH (44).** Following the general protocol, the Wang-resin (900 mg, 0.75 mmol/g) was functionalized. After cleavage with TFA (50% in $CH_2Cl_2$) and evaporation under a stream of $N_2$, the solid was taken up in dioxane and freeze-dried, yielding **44** as a white solid (253 mg, 0.44 mmol, 66%). $^1$H–NMR (400 MHz, DMSO-*D*$_6$) δ 7.36–7.22 (m, 10H, 2 × Ar), 5.11 (s, 2H, $CH_2O$), 4.98 (s, 2H, $CH_2O$), 4.37–4.28 (m, 1H, CαH), 4.11–3.99 (m, 2H, 2 × CαH), 3.41–2.92 (m, 2H, $NCH_2$), 2.88–2.80 (m, 2H, $CH_2CO$), 2.60–2.51 (m, 2H, C≡C$CH_2$), 2.06 (s, 1H, C≡CH), 1.69–1.38 (m, 4H, 2 × $CH_2$); ); HRMS (EI) m/z calcd for $C_{29}H_{34}N_4O_8$ 566.2377, found 566.24065.

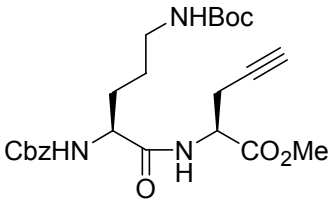


**Fmoc-Orn($N_3$)-hhPrg-OMe (47).** To a suspension of (*S*)-2-amino-6-heptynoic acid (56 mg, 0.39 mmol) in MeOH (5 mL) was added dropwise $SOCl_2$ (75 µL, 1.00 mmol) and the mixture was refluxed for 2h. After cooling to room temperature, the solvents were stripped off yielding the methyl ester **46** as a HCl-salt. The solids were resuspended in $CH_2Cl_2$ (10 mL) and in the following order DiPEA (163 µL, 0.90 mmol), Fmoc-Orn($N_3$)-OH (150 mg, 0.39 mmol) and PyBOP (226 mg, 0.43 mmol) were added upon which the resulting mixture was stirred overnight. After evaporation further purification was performed using flash chromatography (66% EtOAc in heptane), yielding dipeptide **47** (95 mg 0.19 mmol, 47%) as a white solid.

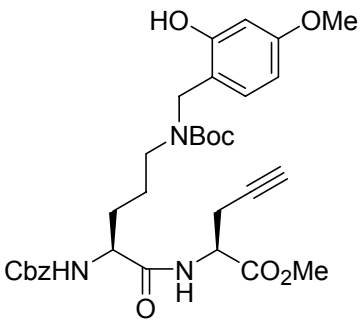
$^1$H−NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.8 Hz, 2H), 7.57 (m, 2H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.30−7.26 (m, 2H), 7.12−7.16 (bd, 1H, NH), 5.88 (m, 1H, NHFmoc), 4.60−4.54 (m, 1H, CαH), 4.44−4.39 (m, 1H, CαH), 4.39−4.32 (m, 2H, $CH_2O$), 4.21−4.18 (m, 1H, CαH-Fmoc), 3.74 (s, 3H, OMe), 3.35−3.22 (m, 1H, $CH_2N_3$), 3.18−3.03 (m, 1H, $CH_2$NHBoc), 2.21−2.16 (m, 2H, C≡C$CH_2$), 2.00−1.50 (m, 8H, 4 × $CH_2$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 172.2, 171.9, 156.1, 143.6, 143.5, 141.0, 127.5, 126.8, 124.9, 119.7, 83.1, 68.9, 66.9, 60.2, 53.3, 52.1, 51.6, 46.9, 39.0, 30.6, 29.9, 26.0, 24.2, 17.7, 14.0; IR ν 3297.7, 3065.5, 2952.7, 2098.2, 1702.9, 1659.3, 1525.2, 1477.9, 1386.8, 1233.8, 1193.4 cm$^{-1}$; HRMS (EI) m/z calcd for $C_{28}H_{31}N_5O_5$ 517.23252, found 517.2301.



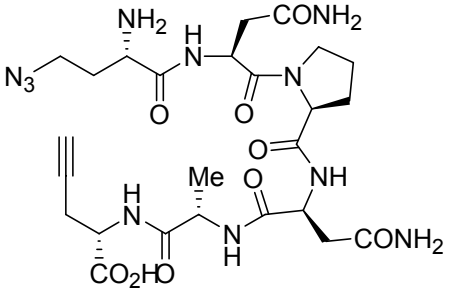

**Cbz-Orn(Orn(N$_3$)Ac)-Prg-OH (49).** Following the general protocol, Wang-resin (600 mg, 0.75 mmol/g) was functionalized. Before cleavage the Fmoc-group was removed and the free amine was capped with $Ac_2O$. After cleavage with TFA (50% in $CH_2Cl_2$) and evaporation under a stream of $N_2$, the solid was taken up in dioxane and freeze-dried, yielding **49** as a white solid (178 mg, 0.33 mmol, 73%). $^{13}$NMR (75.5 MHz, $CD_3OD$) δ 172.5, 172.0, 171.3, 171.0, 156.3, 131.4, 127.6, 127.1, 126.9, 78.2, 70.8, 66.0, 54.1, 52.9, 50.8, 50.4, 38.0, 35.4, 30.1, 29.1, 28.9, 25.1, 24.9, 21.0, 20.8; HRMS (EI) m/z calcd for $C_{25}H_{33}N_7O_7$ 543.2441, found 566.2349 (M + Na)$^+$.



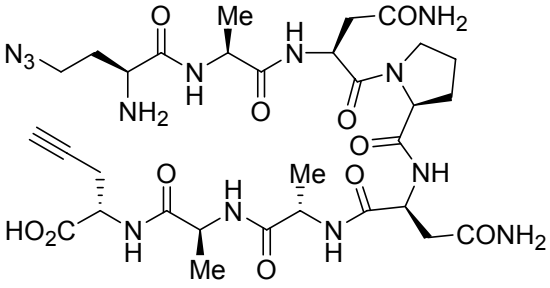

**Cbz-Orn(Boc)-Prg-OMe (59).** To a suspension of (*S*)-2-amino-4-pentynoic acid (200 mg, 1.77 mmol) in MeOH (25 mL) was added dropwise $SOCl_2$ (300 µL, 3.8 mmol) and the mixture was refluxed for 2h. After cooling to room temperature, the solvents were stripped off yielding the methyl ester as a HCl-salt. The solids were resuspended in $CH_2Cl_2$ (20 mL) and in the following order DiPEA (670 µL, 3.94 mmol), Cbz-Orn(Boc)-OH (670 mg, 1.83 mmol) and PyBOP (1 g, 1.85 mmol) were added and the resulting mixture was stirred overnight. After evaporation and further purification using flash chromatography (66% EtOAc in heptane), dipeptide **59** was obtained (750 mg, 1.58 mmol, 88%) as a white solid. $^1$H−NMR (400 MHz, $CDCl_3$) δ7.34−7.29 (m, 5H, Ar), 7.11 (bd, *J* = 7.5 Hz, 1H, NH), 5.69 (bd, *J* = 7.5 Hz, 1H, NHCbz), 5.10 (s, 2H, $OCH_2$), 4.77 (bs, 1H, NHBoc), 4.72−4.67 (m, 1H, CαH-Prg), 4.40−4.35 (m, 1H, CαH-Orn), 3.75 (s, 3H, OMe), 3.24−3.05 (m, 2H, $CH_2N$), 2.75−2.71 (m, 2H, C≡C$CH_2$), 1.99 (t, *J* = 2.6 Hz, 1H, C≡CH), 1.92−1.51 (m, 4H, 2 × $CH_2$), 1.42 (s, 9H, Boc).




**Cbz-Orn(2-hydroxy-4-methoxy-benzyl)-Prg-OMe (60).** Previously obtained dipeptide **59** (750 mg, 1.58 mmol) was dissolved in EtOAc (2 mL) and 2 M HCl (in EtOAc, 10 mL) was added. The resulting mixture was stirred for 45 min and evaporated. The deprotected HCl-salt was then redissolved in $CH_2Cl_2$ (20 mL) and $Et_3N$ (220 µL, 1.58 mmol) was added. The resulting mixture was stirred for 30 min and evaporated. The precipitates were resuspended in dry THF (30 mL) and $Na_2SO_4$ (4.40 g, 31 mmol), 2-hydroxy-4-methoxy-benzaldehyde (240 mg, 1.58 mmol) and $NaHCO_3$ (252 mg, 3.2 mmol) were added, leaving the mixture for several hours to dehydrate (solution turning yellow). When imine formation was completed, $NaB(OAc)_3H$ (2.00 g, 9.6 mmol) was added and the reaction was allowed to stir overnight. The solution became clear/flocky white. The borane remainder was carefully quenched using aqueous saturated ammonium chloride (2 mL) prior to extraction with aqueous saturated $NaHCO_3$ (10 mL) and $Et_2O$ (3 × 50 mL). The remaining oil thus obtained was treated with $CH_2Cl_2$ (10 mL) and $Boc_2O$ (413 mg, 1.91 mmol) for 16 h. Remainder of the $Boc_2O$ was removed with imidazole. Further purification was performed using flash chromatography (3% MeOH in $CH_2Cl_2$), yielding the desired product **60** (222 mg, 0.36 mmol, 23%, 4 steps) as a semi-solid. $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.38−7.33 (m, 5H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.72 (ds, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 6.35 (dd, *J* = 2.4 Hz, 8.0 Hz, 1H), 5.31 (bs, 1H), 5.12 (s, 2H), 4.71−4.67 (m, 1H), 4.25−4.21 (m, 1H), 4.21 (s, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.22−3.17 (m, 2H), 2.77−2.73 (m, 2H), 2.01 (t, *J* = 2.6 Hz, 1H), 1.86−1.79 (m, 1H), 1.63−1.57 (m, 3H), 1.45 (s, 9H). $^{13}$NMR (75.5 MHz, $CDCl_3$) δ 170.8, 170.0, 160.9, 157.0, 155.7, 136.1, 131.9, 128.3, 128.1, 127.9, 115.2, 105.4, 102.3, 81.6, 78.0, 72.0, 67.2, 55.3, 54.4, 52.9, 50.7, 46.8, 46.1, 31.3, 30.1, 28.5, 28.0, 27.4, 23.8, 22.3.




**$H_2N$-hAla($N_3$)-Asn-Pro-Asn-Ala-Prg-OH (51).** Using standard solid phase chemistry (2.31 g, 1.73 mmol) the oligopeptide (723 mg, 1.15 mmol, 67%) was obtained as a white fluffy solid. $^{13}$NMR (75.5 MHz, $D_2O$) δ 173.8, 173.74, 173.3, 173.1, 172.6, 171.2, 170.1, 168.0, 79.0, 71.7, 66.2, 60.7, 52.9, 51.0, 50.9, 50.1, 49.4, 49.3, 48.1, 48.0, 47.9, 47.8, 46.4, 35.9, 35.7, 29.7, 29.2, 24.2, 21.3, 20.5, 16.5; HRMS (EI) m/z calcd for $C_{25}H_{37}N_{11}O_9$ 635.2776, found 658.2623 $(M + Na)^+$.



**$H_2N$-hAla($N_3$)-Ala-Asn-Pro-Asn-Ala-Ala-Prg-OH (52).** Using standard solution phase chemistry on wang-resin (1.71 g, 1.3 mmol) the oligopeptide (700 mg, 0.90 mmol, ~69%) was obtained as a white fluffy solid. HRMS (ESI) m/z calcd for $C_{31}H_{48}N_{13}O_{11}$ 778.35962, found 778.36131 $(M + H)^+$.

# *4.8 References and notes*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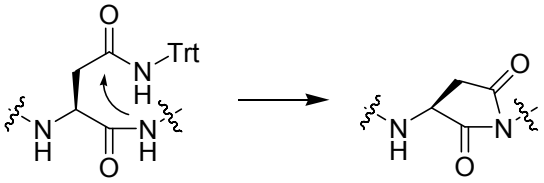


23 The *N*-terminal amino acid is incorporated with the Boc-group since upon cleavage the free amine will be liberated.
24 For an example, see: H. Bieraügel, H. E. Schoemaker, H. Hiemstra, J. H. van Maarseveen, *Org. Biomol. Chem.* **2003**, *11*, 1830.
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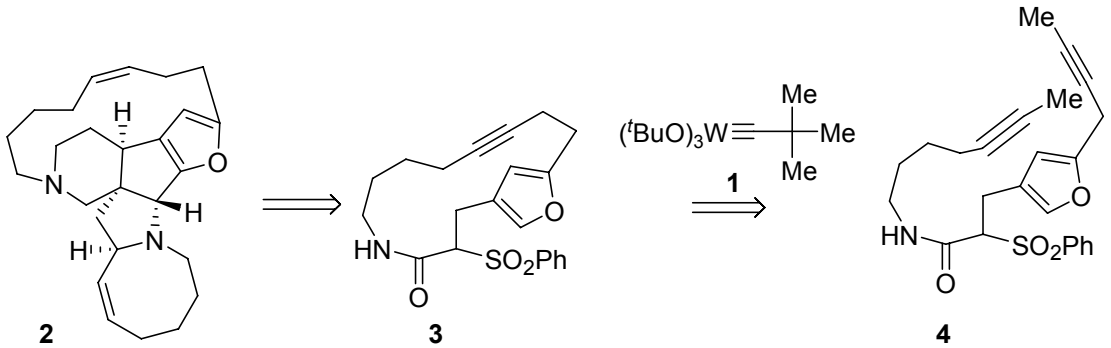
29 A Kaiser test (requiring only a minute amount of resin), involves treatment with two droplets of solutions A, B and C: solution A (ninhydrin (500 mg) in EtOH (10 mL)), solution B (80% phenol in EtOH) and solution C (aqueous 1 mM KCN (2 mL) in pyridine (100 mL)). The resulting mixture is then heated for one min at 100 °C. If a free amine is present, the resin will form a complex which is intensely blue colored (brown for proline).
30 Prg = propargylglycine; hhPrg = bishomopropargylglycine.

# 5 TOWARD ACETYLENE-BASED CYSTINE MIMICS

## *5.1 Introduction*

Compared to the rich history of investigations into olefin metathesis, its alkyne metathesis counterpart has drawn much less attention.[1] Despite the great similarity of the mechanism and the potential transformations, relatively few examples involving alkyne metathesis have appeared in literature pursued by a limited number of research groups. The main features of alkyne metathesis, compared to olefin metathesis, include the inherent absence of (*E*)/(*Z*) mixtures and the surprisingly strong bias for cyclization. These advantages are compensated by the relative sensitivity and difficult accessibility of suitable catalysts. Since general acceptance of new methodology usually very much depends on its ease of use, alkyne metathesis may only become a widely used tool in case commercially available, air-stable catalysts emerge. The latter situation was encountered in the olefin metathesis field, which reached wide acceptance with the emergence of the 'air-stable' 1st generation Grubbs catalyst in 1995,[2] while Schrock's superior, but air-labile catalyst was already available since 1990.[3]

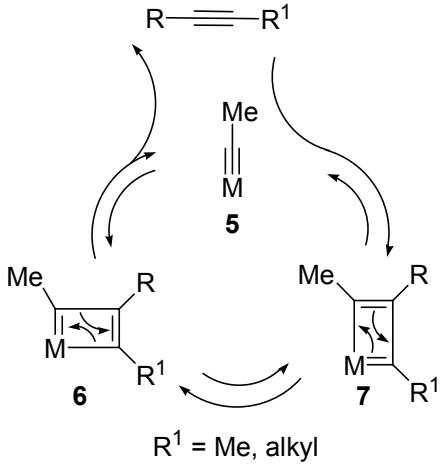
Nevertheless, recent developments culminated in 1999 in the first example of a total synthesis based upon ring-closing alkyne metathesis (RCAM) from the Fürstner group, being the synthesis of nakadomarin (**2**) (Scheme 1).[4]




**Scheme 1**. *Retrosynthesis of nakadomarin, featuring RCAM as the key step.*

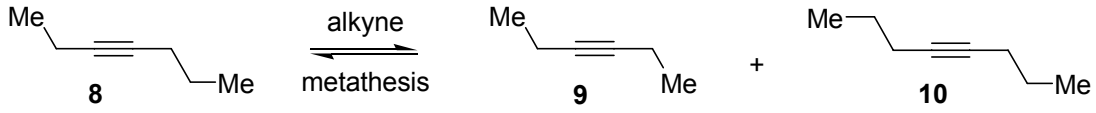
The earliest reports of alkyne metathesis date from the late 1960s,[5] when heterogeneous catalysts (e.g. silica-bound tungsten oxide) catalyzed alkyne

metathesis reactions at 400 °C.[6] During these early days, especially olefin metathesis was drawing much attention in the chemical community in terms of application, suitable catalysts and mechanism. In analogy with the proposed Chauvin mechanism for olefin metathesis,[7] Katz and coworkers came up with a similar mechanism for alkyne metathesis,[8] now involving alkylidyne complexes (Scheme 2). This mechanism implies that initiators for metathesis should be found among alkyl-substituted metal-carbyne complexes (**5**) and that the catalytic cycle involves the formation and ring-opening of metallacyclobutadiene species.




**Scheme 2**. *Mechanism of alkyne metathesis as proposed by Katz.*

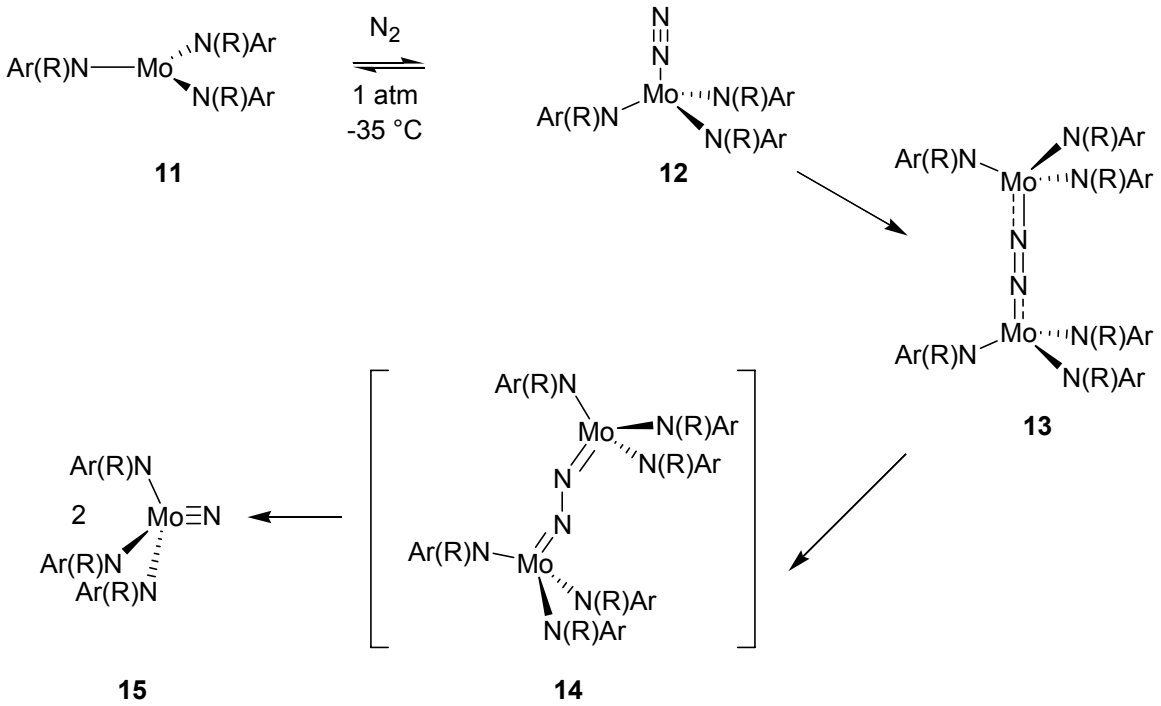
It was not until well-defined metal alkylidyne complexes such as [($^t$BuO)$_3$W≡CCMe$_3$] (**1**) became available, that the first alkyne metathesis reactions were successfully carried out, thus confirming the mechanistic hypothesis. The Schrock group was the first to report the synthesis of the tungsten-catalyst **1** and demonstrated its application in the cross-metathesis of 3-heptyne, as shown in Scheme 3.[9]




**Scheme 3**. *Alkyne metathesis-mediated exchange of alkyl substituents.*

In these early examples, it was already observed that only internal acetylenes (RC≡CR$^1$) successfully undergo the alkyne metathesis process, whereas terminal acetylenes (RC≡CH) are much more prone to undergo polymerization reactions and therefore cannot be used in alkyne metathesis.[10] Moreover, it was observed that alkyne metathesis catalysts displayed excellent chemoselectivity for alkynes and did not react with olefins in the molecule.[11]

The group of Fürstner further developed the scope of RCAM and initiated a program to search for alternative catalysts in the late nineties.[12] As a result, sterically hindered trisamidomolybdenum(III) complexes of the general type [Mo{($^t$Bu)(Ar)N}$_3$] (**11**) were found to react with CO, NO, $N_2O$ and also $N_2$ in a stoichiometric fashion (Scheme 4).[13] Due to this remarkable reactivity, the Fürstner group set out to apply species of type **11** in alkyne metathesis. Although complex **11** was inactive on test substrates, the addition of $CH_2Cl_2$ or other halogen sources (TMSCl) resulted in an efficient and rapid alkyne metathesis reaction. This novel catalyst, when activated, outperformed existing protocols in many respects, independent of its use in RCAM, homo-alkyne dimerization or cross-alkyne metathesis.[14] However, drawbacks remain such as sensitivity to oxygen, moisture and acidic protons of e.g. alcohols and acids. Even secondary amide protons suffice to inactivate the catalyst, rendering it useless for application of RCAM in for example peptides.




**Scheme 4**. *Reaction of molybdenum-catalyst **11** with molecular nitrogen.*

As mentioned before, despite the broad scope of ring-closing metathesis (RCM), ring-closing alkyne metathesis has specific advantages over olefin metathesis, especially the lack of formation of geometrical isomers. This is exemplified by RCM examples in the synthesis of carbocyclic analogues of cyclic peptides, where generally mixtures of (*E*)- and (*Z*) isomers are obtained.[15] These carbon analogues

are generally designed as synthetic mimics to attain a metabolically (and chemically) stable ß-turn peptide as compared to nature's disulfide bridges.
As shown in Section 1.4, Grubbs successfully demonstrated the application of RCM for the formation of carbon analogues of cystine.[16] Generally these are made by incorporation of two unsaturated amino acids in peptides and subsequent metathesis.

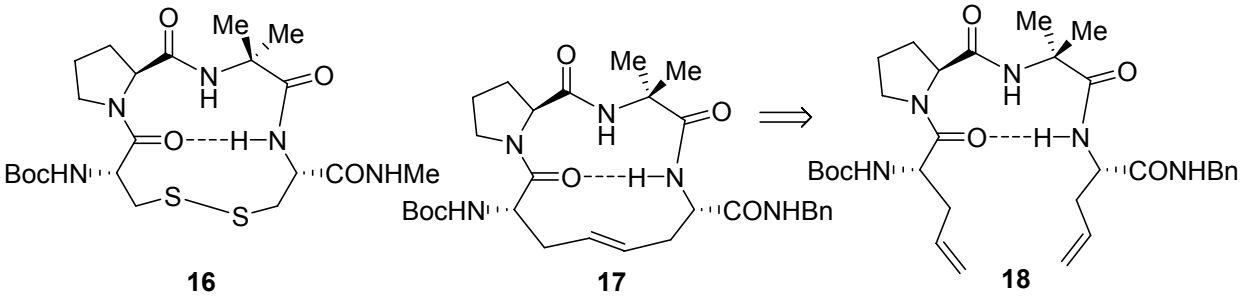
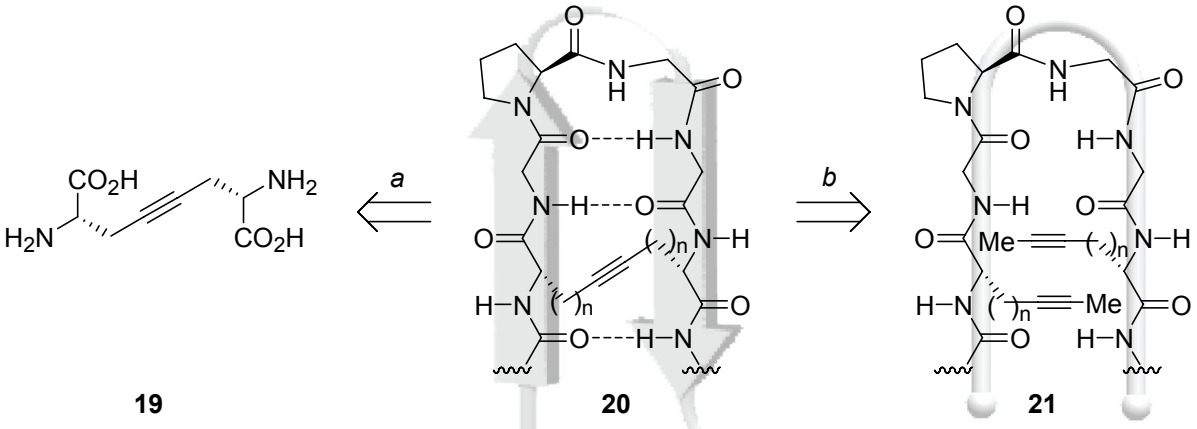



**Figure 1**. *Balaram's tetrapetide (**16**) and Grubbs carbon mimic (**17**)*

In Chapter 3 has already been indicated that we were interested in the synthesis of acetylene-bridges as stable cross-links in peptide units. This can either be achieved *via* incorporation of an acetylene-containing diaminosuberic acid fragment in a peptide, or via incorporation of monomeric RCAM precursors in a peptide, followed by RCAM after the peptide has been synthesized. While the former approach (Scheme 5, path *a*) has been detailed in Chapter 3,[17] we now wish to detail the latter pathway.



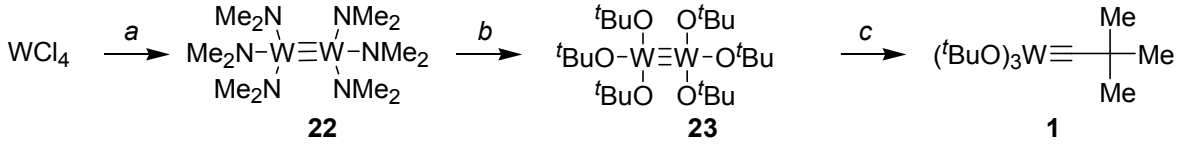

**Scheme 5**. *Fixation of a peptide β-turn via an unsaturated all carbon cross-link.*

The use of RCAM for peptide chemistry was already demonstrated in our group by Wolf (see Section 3.1).[18] Using this approach by building peptides containing unsaturated amino acids (*viz.* **21**) and applying RCAM (Scheme 5, path *b)*, we intend to make constrained peptides (**20**).[19] Interestingly, this approach was later also pursued by the Liskamp group. [20]

These general studies to employ acetylene-containing amino acids and ring-closing alkyne metathesis as an approach to prepare stable ß-turn elements involves the synthesis of the RCAM catalyst, the synthesis of suitable oligopeptides with varying length and motifs and a study of the conformational behavior of the ß-turn-containing peptides.

## 5.2 Synthesis of an RCAM catalyst

Since alkyne metathesis catalysts are not commercially available, we set out to prepare the tungsten-catalyst **1** following the published procedures (Scheme 6).[21] The synthesis commenced with freshly prepared lithium dimethylamide, which was reacted at –60 °C with tungsten(IV) chloride. After warming up to room temperature and additional refluxing for two hours, evaporation and subsequent vacuum sublimation yielded **22** as red-brown crystals.




**Scheme 6.** *Reagents and conditions: a) $LiNMe_2$, THF, –60 °C to rt (44%); b) $^tBuOH$ (66%); c) neopentyne, heptane (44%).*

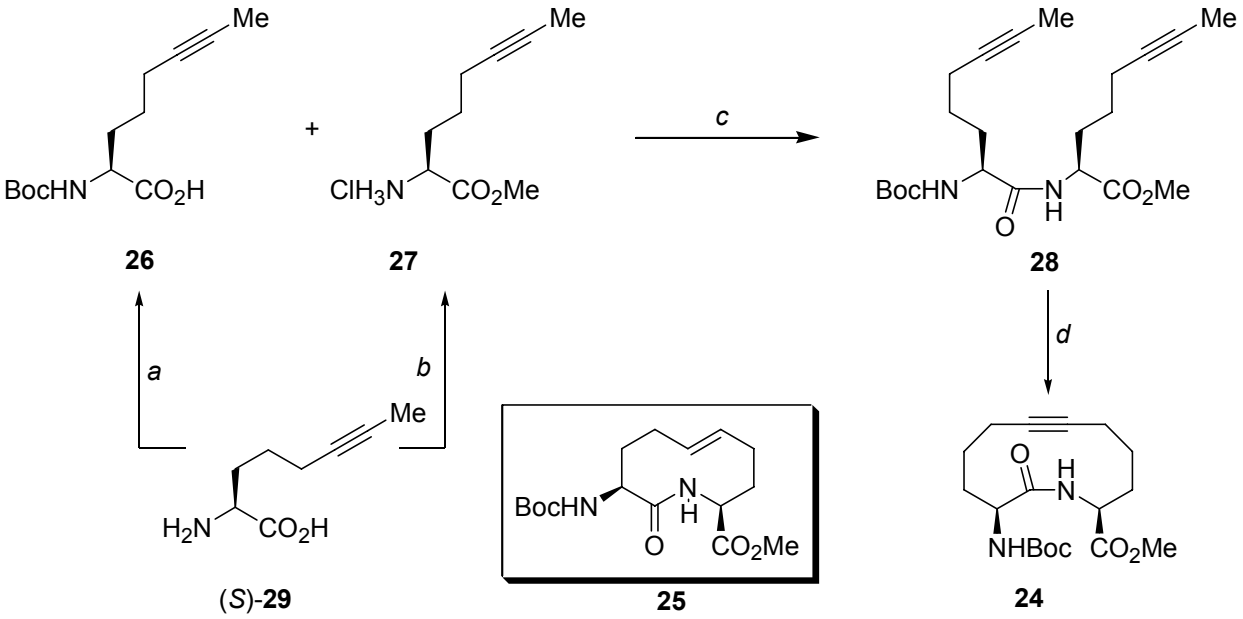
Then, ligand exchange with *tert*-butanol and subsequent recrystallization in heptane yielded the complex **23**.[22] This compound was subjected to neopentyne[23] to cleave the dimeric species, yielding catalyst **1** which was obtained in pure form by sublimation. Due to the fact that the catalyst readily decomposes at elevated temperatures, a low pressure was required ($T_{sub}$ = 45 °C at 0.01 mbar). Eventually, catalyst **1** was obtained as clear pale yellow crystals, which were collected from the sublimation condensor in a glove box in an overall yield of 13%.

## 5.3 Benchmark studies

Given the delicacy of the RCAM process and our initial lack of experience with this particular catalyst and reactions, we were looking for a conversion that would allow us to benchmark our results. A suitable target reaction we envisioned, was the cyclization of dipeptide **28** to the cycloalkyne **24**, which is an acetylene variant of the cyclic dipeptide **25** (Scheme 7). Katzenellenbogen had previously demonstrated that this olefin acts as a ß-turn mimic,[24] and

preliminary results on this particular cyclization had already been obtained in our lab.[25]

The precursor synthesis commenced with enantiomerically pure (*S*)-2-amino-6-octynoic acid (**29**), which was either protected at the nitrogen atom with a Boc group (**26**) or reacted at the acid function to give the corresponding methyl ester **27**. Both amino acids were then coupled via a mixed anhydride to form the dipeptide **28** in 87% yield.

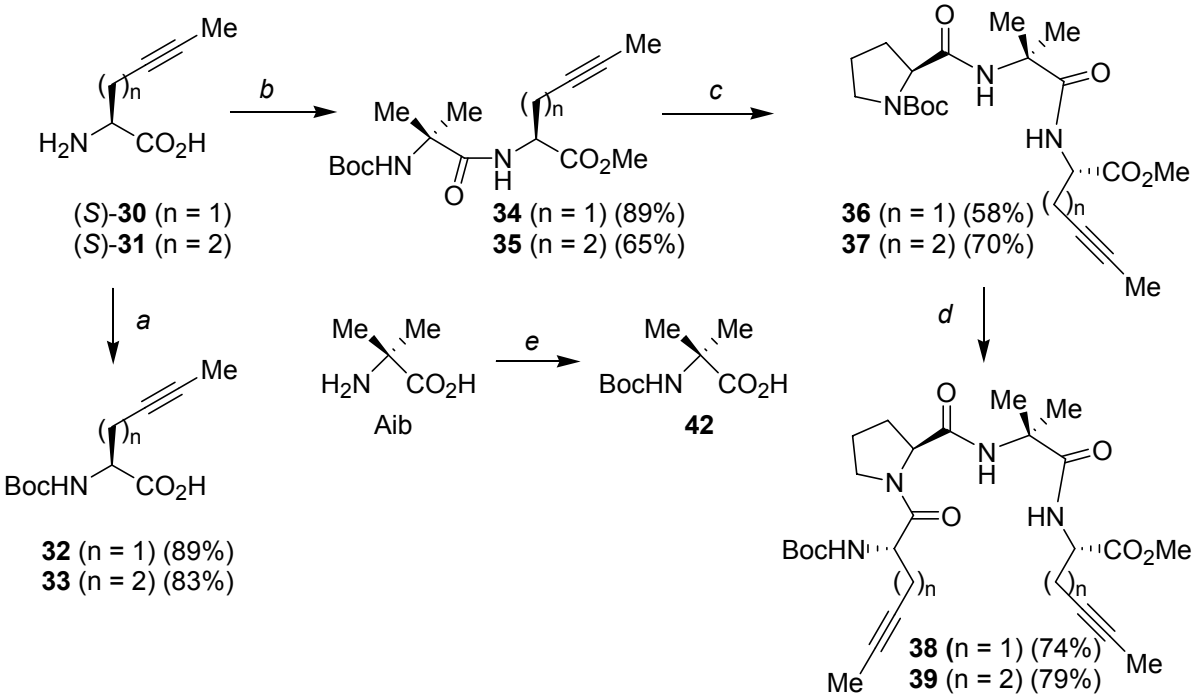



**Scheme 7.** *Reagents and conditions; a) $Boc_2O$, $NaHCO_3$, 1,4-dioxane/$H_2O$ (1 : 1), 100 °C (85%); b) $SOCl_2$, MeOH, reflux (100%); c) $^{i}BuOCOCl$, $Et_3N$, $Et_2O$, $CH_2Cl_2$, 0 °C (87%); d) PhMe,* **1**, *80 °C, 3 h (72%).*

This RCAM precursor was now subjected to the ring-closing metathesis conditions (10% of **1**, toluene, 80 °C, 3 h) to give the desired cycloalkyne **24** in 72% yield. Generally these results were found to be reliable giving yields between 70 and 80%. This gratifying result clearly demonstrates the potential of ring-closing alkyne metathesis, even in combination with highly functionalized substrates such as these amino acids. Furthermore, this result encouraged us to investigate ring-closing alkyne metathesis in larger peptide systems.

## *5.4 Tetramer cyclization*

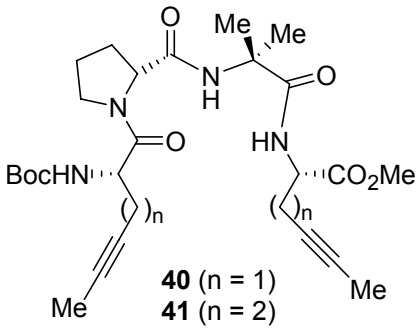
Having established a reliable protocol for RCAM reactions on dipeptides, we now set out to apply this reaction on larger structures. Initially, we chose to work with tetrapeptides that we would like to stabilize via the formation of acetylene linkages.[26] We deliberately chose to work with solution phase strategies, since for the desired length (four to five amino acid residues) this is more suitable to prepare gram amounts of material. The sequence commenced with (*S*)-2-amino-4-hexynoic acid (**30**), which was treated with MeOH under acidic conditions to form the corresponding methyl ester. Next, it was reacted as a free amine with Boc-Aib-OH (**42**)[27] in the presence of Castro's reagent (PyBOP)[28] and DiPEA in $CH_2Cl_2$ to give **34** in 89%. Alternating Boc deprotection using hydrochloric acid in EtOAc and the previously described coupling protocol involving subsequently Boc-protected proline and **32** gave eventually the fully protected tetrapeptide **38** in 38% overall yield after silica gel flash chromatography.




**Scheme 8**. *Reagents and conditions: a) $NaHCO_3$, $Boc_2O$, 1,4-dioxane/$H_2O$ (1 : 1), 100 °C; b) i: $SOCl_2$, MeOH ii: PyBOP, DiPEA,* ***42****, $CH_2Cl_2$; c) i; 2 M HCl in EtOAc, 45 min ii: PyBOP, DiPEA, Boc-Pro-OH, $CH_2Cl_2$ (58%); d) i; 2 M HCl in EtOAc, 45 min ii: PyBOP, DiPEA,* ***32*** *or* ***33****, $CH_2Cl_2$, e) 2 M NaOH, $Boc_2O$ (65%).*

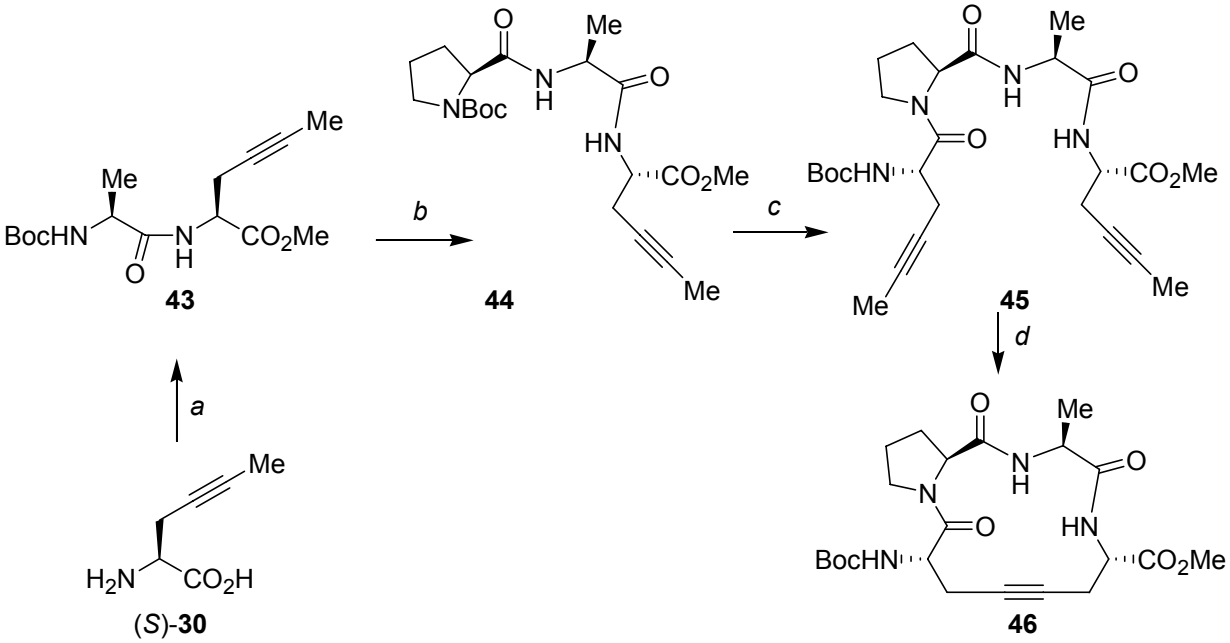
RCAM of **38** using tungsten-catalyst **1** (9 mol%, chlorobenzene or toluene, 80 °C, 3 h)[29] did not result in the desired cyclic peptide. While the corresponding olefin derivative is known to readily cyclize,[30] this specific tetrapeptide failed to undergo cyclization despite numerous attempts using a variety of conditions.

Alternative sequences (made according to Scheme 8) based on elongated acetylene handles using (*S*)-2-amino-5-heptynoic acid (**39**) or the (*R*)-instead of (*S*)-proline-containing diastereomers **40** and **41** also never gave rise to any cyclized products. Most likely, dimers, or oligomers were formed instead via intermolecular pathways since the acetylenic methyl groups (δ ~1.74, t, $J$ = 2.4 Hz) were clearly consumed according to $^1$H−NMR. Presumably, the Pro-Aib-based turn sequence is too rigid to allow approach of the two acetylenes in a manner suitable to effect cyclization.



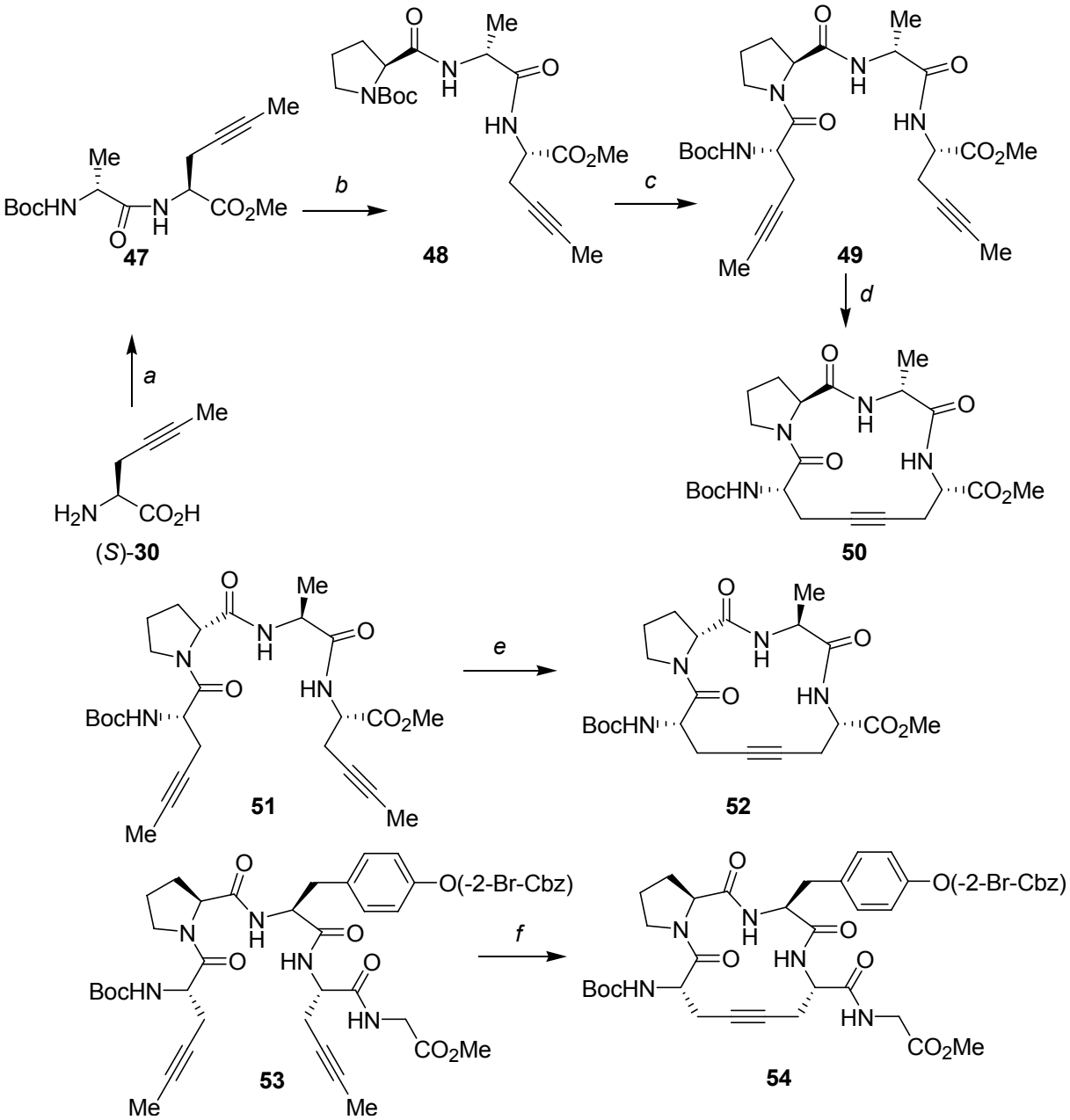

**40** (n = 1)
**41** (n = 2)

In order to reduce the rigidity of the system, similar tetramers were synthesized containing alanine residues instead of Aib. For example, in an alternative synthetic sequence (Scheme 9), the (*S*)-alanine-based tetrapeptide **45** was synthesized in six steps in an overall yield of 62%.




**Scheme 9**. *Reagents and conditions: a) i: $SOCl_2$, MeOH ii: PyBOP, DiPEA, Boc-Ala-OH, $CH_2Cl_2$ (59%); b) i; 2 M HCl in EtOAc, 45 min ii: PyBOP, DiPEA, Boc-Pro-OH, $CH_2Cl_2$ (62%);c) i;2 M HCl in EtOAc, 45 min ii: PyBOP, DiPEA,* ***32****, $CH_2Cl_2$ (76%), d) PhMe,* ***1****, 80 °C, 3 h (72%).*

Upon application of RCAM on this tetrapeptide using 10% of catalyst **1** in toluene at 80 °C, the reaction was finished within one hour. In the same fashion, (*R*)-alanine derivative **49** and (*R*) Proline derivative **51** were constructed (Scheme 10) and subjected to initial RCAM conditions, resulting in similar recoveries of the cyclized diastereoisomers **50** and **52**. Similarly, the pentapeptide **53** was transformed into cyclic compound **54**, albeit in only 36% yield.




**Scheme 10.** *Reagents and conditions: a) i: $SOCl_2$, MeOH ii: PyBOP, DiPEA, Boc-(R)-Ala-OH, $CH_2Cl_2$ (51%); b) i; 2 M HCl in EtOAc, 45 min ii: PyBOP, DiPEA, Boc-Pro-OH, $CH_2Cl_2$ (57%); c) i; 2 M HCl in EtOAc, 45 min ii: PyBOP, DiPEA,* ***32****, $CH_2Cl_2$ (76%), d) PhMe,* ***1****, 80 °C, 3 h (70%); e) PhCl,* ***1****, 80 °C, 3 h (57%); f) PhCl,* ***1****, 80 °C, 3 h (36%).*

Since both (*R*)- and (*S*)-alanine-based tetramers successfully underwent cyclization and the amino isobutyric acid derivatives did not, spatial distortion can be ruled out as plausible reason for the failure of cyclizing **38-41**. Presumably, the Aib-Pro sequence gives a too rigid ß-turn and thus prevents successful RCAM. This is remarkable, since RCM on its olefin counterpart was successful.[30]

# *5.5 Conformational NMR-studies*

Upon cyclization of the tetrapeptides, a constrained peptide is formed. This can be observed with $^1$H−NMR, in which apart from the disappearance of the methyl groups, all α-protons and amide protons dramatically shift due to mutual interactions in the ring system (Figure 3).

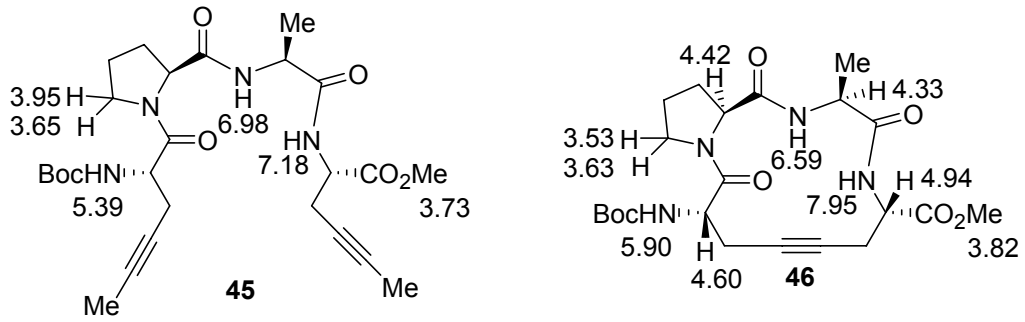



**Figure 2.** *Chemical shifts.*

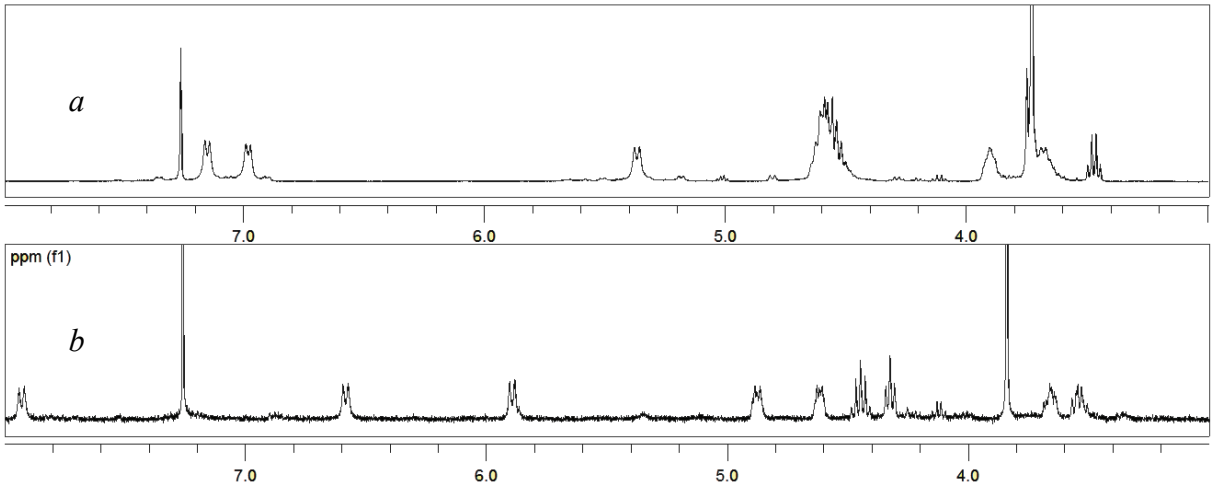



**Figure 3**. *Selected region of the $^1$H−NMR spectrum before (a) and after (b) ring closure of compound **45**.*

At this stage, compounds **46**, **50** and **54** were analyzed by $^1$H−NMR and compared with their cystine-containing counterparts **55** (Figure 2). The precursor of the latter cyclic peptide was readily prepared following peptide synthesis protocols as outlined for the synthesis of the linear peptides, but employing suitably protected Cystiene derivatives instead of (*S*)-2-amino-4-hexynoic acid derivatives ((*S*)-**30**). Oxidation of the Cysteine residues was readily accomplished under standard conditions ($I_2$, MeOH, DMF, ascorbic acid, citrate buffer) to afford the target cyclic peptide **55** in 55% overall yield.

Relevant $^1$H−NMR NOE signals observed for the different peptides are depicted in Figure 4. Comparison of the NOESY spectra of 54 and 55 revealed several similarities. In both cases $H_c/H_d$ interactions were present, indicating a *trans*-

amide bond at this side of the molecules. Furthermore, NOEs between $H_a/H_b$ and $H_b/H_e$ suggest a ß-turn as the major structural motif for both protected peptides.

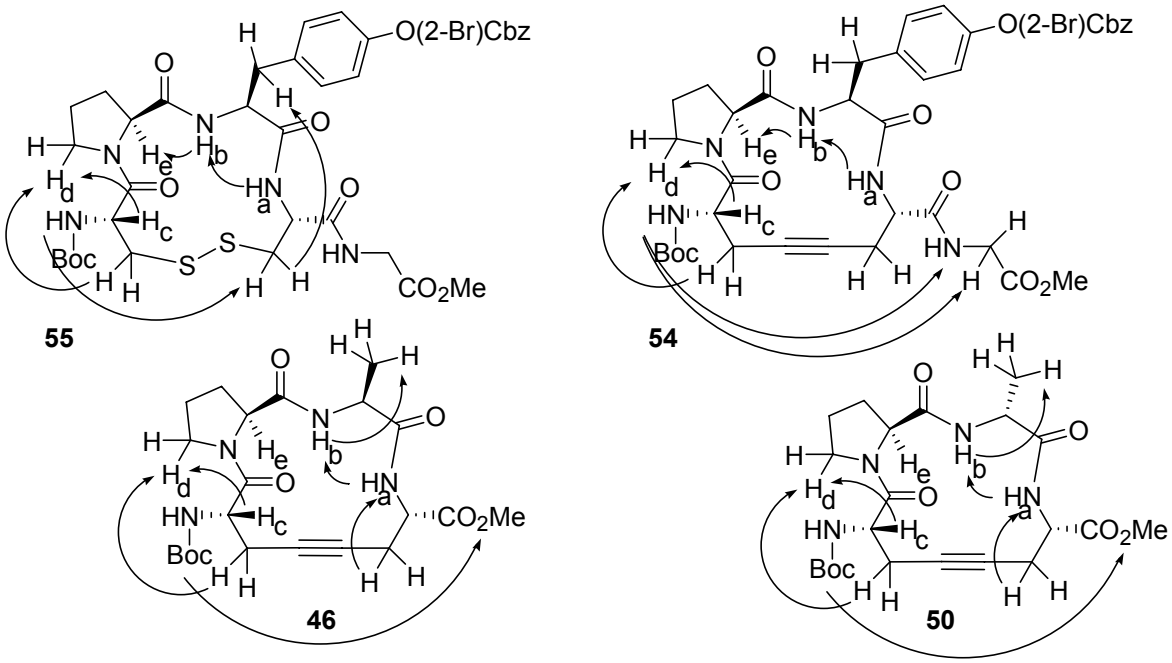



**Figure 4.** *$^1$H–NMR-correlations.*

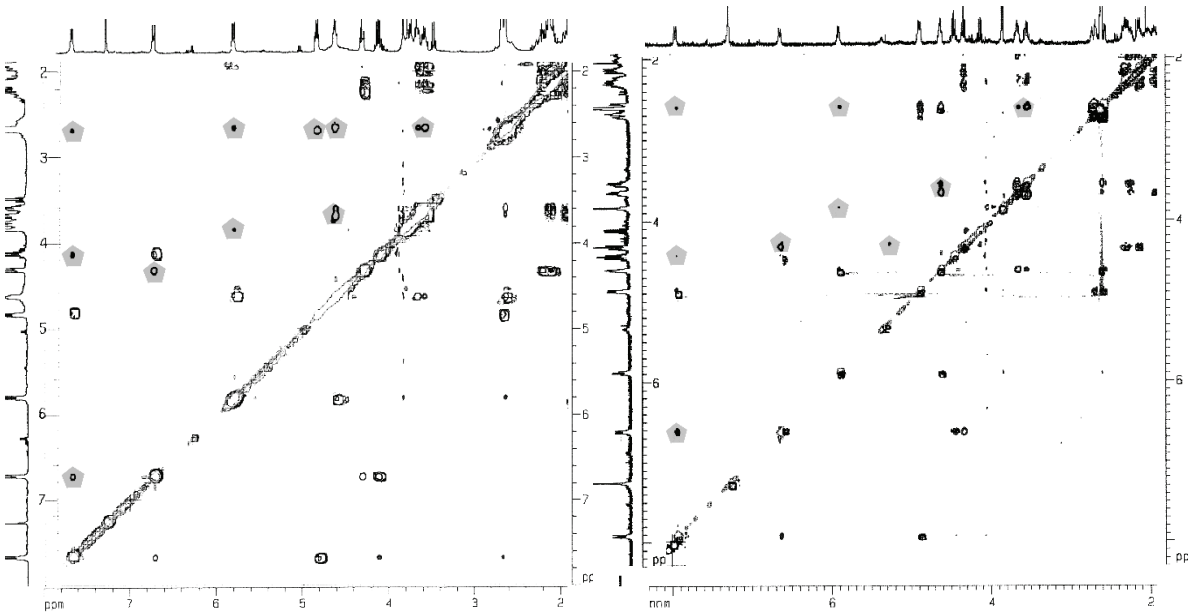


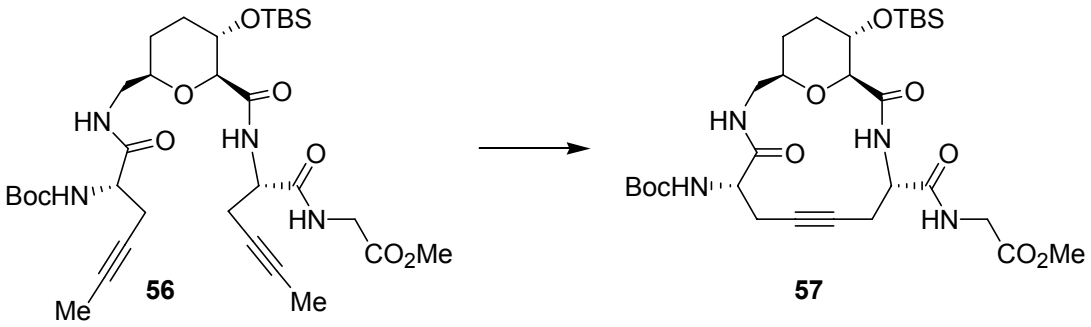
**Figure 5.** *ROESY's of RCAM-products Boc-Mpg[31]-Pro-Ala-Mpg-OMe **46** (left) and its diastereoisomer **50** (right). Markings indicate the additional correlations revealed by ROESY overlaying COSY.*

The most remarkable difference entails the presence of an NOE between NH-Boc and the NH- and α-protons of the glycine residue in **54**, all of which are absent in cystine analogue **55**. Presumably, the rigid acetylene bridge forces these protons to be in close proximity. The methyl ester of the shorter tetramers **46** and **50** also

correlates to the NH-Boc, which confirms the closer proximity relative to the cystine-bridged peptides.
Attempts to study the involvement of amide protons in H-bonding through either temperature-dependent chemical shift studies (spectra were recorded in DMSO-*d6*) or solvent titration studies were abortive for both **54** and **55** due to overlap of the amide signals and the aromatic protons.
These structural analyses demonstrate that replacement of a disulfide bridge with an acetylene moiety renders the cyclic peptides more rigid, favoring additional interstrand proton-proton interactions.

## *5.6 Alternative β-turn mimics*

The presence of ß-turns is one of the factors responsible for ß-sheet formation. New ß-turn inducing moieties have emerged in literature during the past few years. Examples of these mimics are the aforementioned cyclic dipeptide **24** by Katzenellenbogen, but also backbones based on the sugar derivative **56** (Scheme 11) are known to act as a ß-hairpin inducer.[32] In a preceding project was shown how a sugar-based ß-turn motif, functionalized with two 2-amino-hex-4-ynoic acid (**30**) units, can undergo RCAM to form **57**.




**Scheme 11.** *Reagents and conditions: PhCl, 1, 80 °C (25%).*

However, these sugar amino acid moieties are obtained after rather extensive synthetic sequences and therefore less convenient for use as a ß-turn inducer. In contrast, a recent paper by Nowick described the use of commercially available ornithine as a ß-turn inducer (**59**) and compared it to the (*R*)-Pro-Gly moiety (**58**).[33] The latter moieties, a well-known ß-turn inducer, were incorporated in several ß-hairpin sequences based on Arg-Trp-Gln-Tyr-Val-*Ava*-Lys-Phe-Thr-Val-Gln-NH, replacing Ava at the ß-turn position.[34]

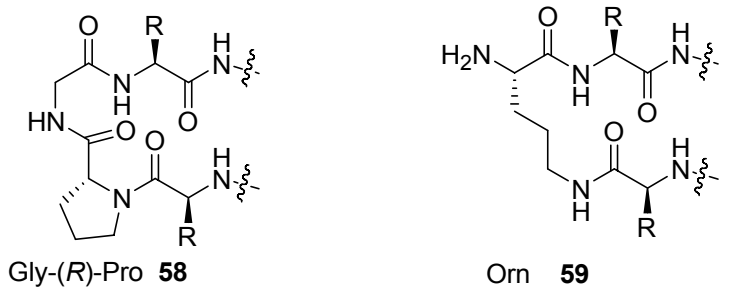
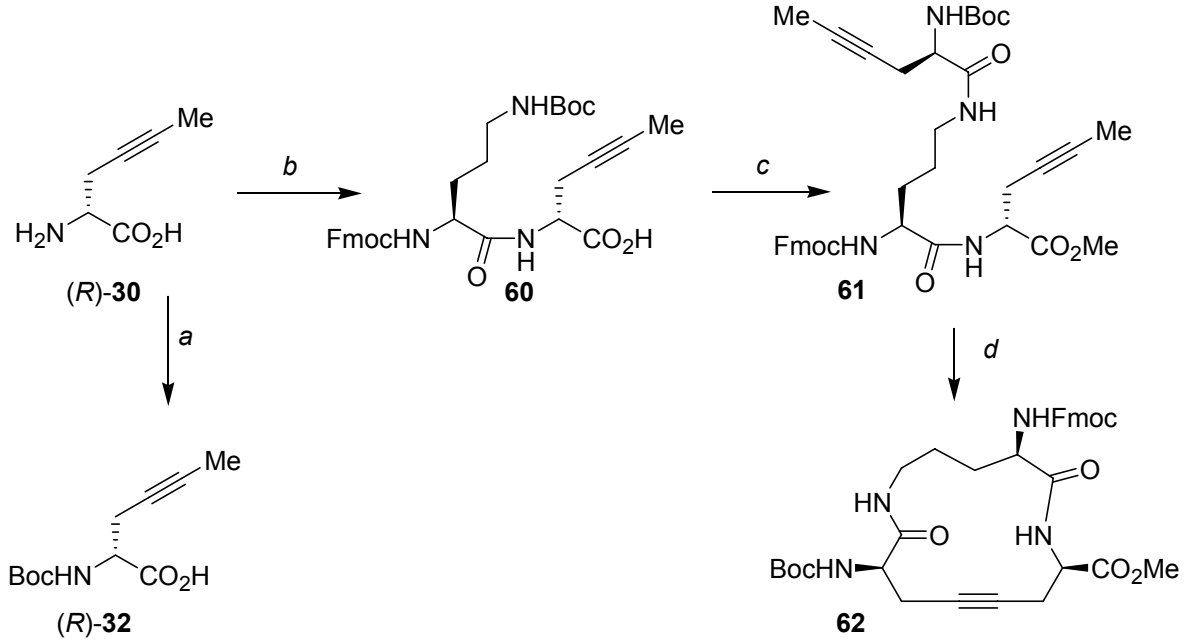



**Figure 6**. *Ornithine as β-turn inducer as compared to Gly-(*R*)-Pro.*

The results showed that replacement led to only little deviation for the amide protons. This strongly suggests that the ornithine-induced turn (*viz.* **59**) is comparable to the (*R*)-Pro-Gly (*viz.* **58**) turn in promoting ß-hairpin formation in peptides. Apart from the fact that a shorter peptide synthesis is required, it also provides an additional functional group for further functionalization, rendering this approach rather attractive.




**Scheme 12.** *Reagents and conditions: a) $NaHCO_3$, $Boc_2O$, 1,4-dioxane/water (1 : 1), 100 °C (99%); b) i: $SOCl_2$, MeOH, reflux; ii:) Fmoc-Orn(Boc)-OH, PyBOP, DiPEA, $CH_2Cl_2$; c) i: 2M HCl in EtOAc, 45 min; ii PyBOP, DiPEA, (*R*)-**32**, $CH_2Cl_2$ (24%, 4 steps); d) PhMe, **1**, 80 °C, 3 h (38%).*

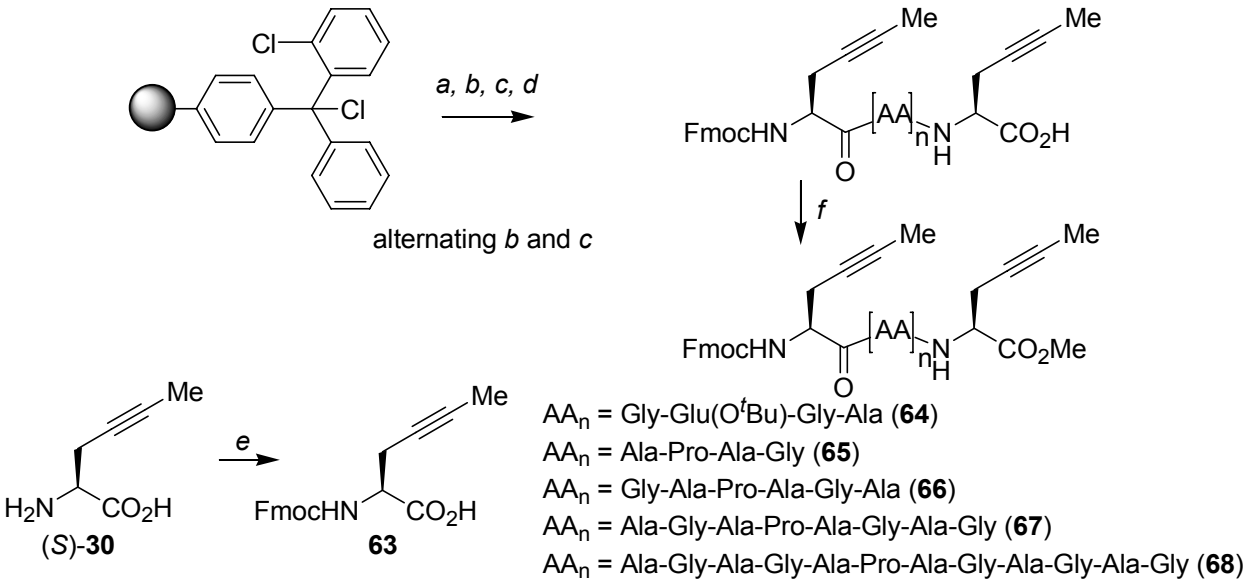
Orthogonal protection of both ornithine amine functional groups allows selective coupling on the side chain. Initial condensation using Castro's reagent in presence of DiPEA with suitably protected (*R*)-**30** with Fmoc-Orn(Boc)-OH was followed by selective acidic (hydrochloric acid in EtOAc) deprotection of the *side chain* protecting Boc-group. After the second condensation using Castro's reagent and DiPEA with Boc-protected (*R*)-2-amino-hex-4-ynoic acid ((*R*)-**32**) tripeptide **61** was made in an overall yield of 24%. Ring-closing alkyne metathesis using

standard conditions (10 mol% catalyst, toluene, 80 °C) gave rise to the cyclized product **62** in reasonable yield. This system could provide an interesting handle for future peptide systems. It must be noted however that the absence of the internal hydrogenbond leads to a more flexible turn.

## *5.7 Larger oligopeptides*

Having established reliable RCAM protocols for the synthesis of tetrameric and pentameric cyclic peptides, we wished to extend this methodology for the synthesis of more extended acetylene cross-linked ß-hairpin structures. Because for this purpose solution phase synthesis would become too laborious, we switched to solid phase strategies, which can easily provide larger oligopeptide structures. We showed previously that RCAM could not be successfully performed on the resin, so we chose to do this crucial cyclization reaction after cleavage from the resin.

A solid phase peptide synthesis procedure was designed aimed at providing a small library of peptides of variable length, with each member containing acetylene residues on both ends. We chose to work with a 2-chlorotrityl-functionalized resin due to the well-known very mild cleavage conditions, which would allow side chain protection. Using Fmoc-protection (*S*)-**30** was efficiently protected in 96% to yield the desired building block **63** for solid phase chemistry peptide synthesis.




**Scheme 13.** *Reagents and conditions: a) $CH_2Cl_2$, Fmoc-(S)-2-amino-hex-4-ynoic acid (63); b) DIC, HOBt, Fmoc-AA-OH, DMF; c) 0.1 M HOBt, 20% piperidine in DMF; d) 1% TFA in $CH_2Cl_2$; e) FmocOSu, $Et_3N$, $CH_3CN$, $H_2O$ (96%); f) $CH_2N_2$ in $Et_2O$ or $SOCl_2$ in MeOH.*

A modest oligopeptides-library was thus synthesized and after cleavage protected as the corresponding methyl esters using either diazomethane or mildly acidic MeOH.[35] Characterization using MALDI-TOF mass spectroscopy and $^{13}C$–NMR spectroscopy confirmed the successful synthesis of final oligopeptides. The spacer between the ß-turn and acetylenic amino acids consist of alternating alanine and glycine residues. These sequences are often encountered in ß-sheet motifs present in spider silk and are also chemically inert.[36]

Attempts to apply our previously developed RCAM conditions on these substrates revealed a major drawback of larger peptides. Virtually all of these larger peptides were very poorly soluble in toluene. Several attempts such as preheating of the peptide in the solvent, applying a high dilution and using halogenated solvents as co-solvent were to no avail, since in any of these cases no RCAM products could be observed. This led us to conclude that RCAM is only suitable for small oligopeptides (up to five amino acids) and that alternative approaches are required for larger peptides.

## *5.8 Conclusions*

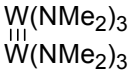
In this chapter an overview of ring-closing alkyne metathesis as a tool to introduce conformational restriction in peptides has been given. Fairly straightforward construction of dipeptide, tripeptide and tetrapeptides equipped with unsaturated amino acids and subsequent RCAM under inert conditions gave rise to good yields. Using 2D NMR-spectroscopy conformational features of the resulting products were measured and the acetylene bridge was confirmed to give a more rigid turn than a comparable cystine-based system. Extending RCAM to larger oligopeptides gave problems with the nature of peptide-systems. Solubility problems with inert solvents and diminished activity due to chelating effects of the peptide around the transition metal could not be overcome. This obviously is a limiting factor in such applications of RCAM.

## *5.9 Acknowledgements*

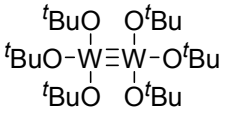
We thank Dr. Bing Wang (DSM Research, Geleen, The Netherlands) for his assistance with the synthesis of the RCAM catalyst and DSM Geleen for its hospitality. Dr. Begoña Aguilera and Dr. Mark Overhand are kindly acknowledged for the synthesis of the cystine tetramers **53**-**55** and sugar-based ß-turn **56** and **57**. Ad Swolfs is acknowledged for his assistance with the NMR-techniques.

# *5.10 Experimental section*

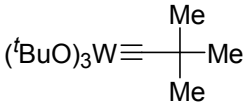
For general experimental details, see: Section 2.8.



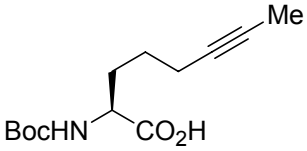
**$(NMe_2)_3W{\equiv}W(NMe_2)_3$ (22).** Using Schlenk conditions, dimethylamide (50.0 mL, 2 M in THF, 100 mmol) was cooled to –40 °C and BuLi (58.1 mL, 1.6 M in hexane, 93.8 mmol) was added dropwise. After raising the temperature to room temperature and stirring for 18 h, the mixture was evaporated *in vacuo*. The resulting white solids were dissolved in THF (60 mL), cooled to –60 °C and transferred to a suspension of $WCl_4$ (7.37 g, 23.3 mmol) in $Et_2O$ (at 0 °C). The reaction was allowed to warm to room temperature and stirred for 18 h. After additional refluxing for 2 h, the mixture was evaporated and the resulting brown solid was transferred in a glove box to a sublimation flask for further purification. Sublimation (110 °C, 0.01 mbar) yielded **3** as a red-brown solid (3.24 g, 6.1 mmol) in 44% yield.



**($^t$BuO)$_3$W≡W(O$^t$Bu)$_3$ (23).** Using Schlenk conditions, tungsten hexa(dimethylamine) (3.23 g, 6.10 mmol) was dissolved in KBP[37] 40 mL) and freshly distilled $^t$BuOH (20 mL) was added. After stirring for 16 h, the dark red mixture was evaporated *in vacuo*. The darkbrown solids were dissolved in KBP (40 mL) and concentrated to approximately 20 mL so that most of the compound precipitated. Upon heating, all material dissolved again and after standing for 18 h at –30 °C a large precipitate was formed. The solvent was removed using a canula and the remaining solids were dried using high-vacuum, yielding dark red crystals (2.70 g, 3.35 mmol, 66%).

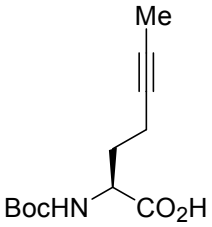


**($^t$BuO)$_3$W≡C$^t$Bu (1)**.[38] Using Schlenk conditions, tungsten hexa-*tert*-butanol (2.70 g, 3.35 mmol) was dissolved in KPB (100 mL) and freshly distilled and degassed 4,4-dimethyl-2-pentyne was added dropwise. The reaction was stirred for 16 h and evaporated *in vacuo*. Further purification using sublimation (45 °C, 0.01 mbar)[39] yielded faint yellow crystals (1.41 g, 3.06 mmol) in 44% yield.

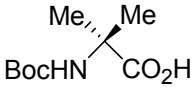


**(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-6-octynoic acid (26).** To a solution of (*S*)-2-amino-oct-6-ynoic acid (2.00 g, 12.9 mmol) in dioxane/water (1/1, 150 mL) sodium bicarbonate (2.16 mg, 25.1 mmol) and $Boc_2O$ (4.28 g, 19.6 mmol) were added. The

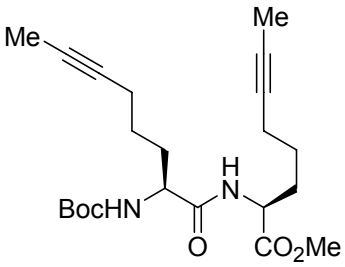
reaction mixture was heated at reflux temperature for 2 h. The dioxane was evaporated and the aqueous layer was acidified with 2 M HCl and extracted using EtOAc (3 × 75). The organic layer was washed (brine,150 mL), dried ($MgSO_4$) and evaporated. After purification of the crude product using flash chromatography (85% EtOAc, 1% AcOH in heptane), Boc-hhMpg-OH **26** (2.19 g, 8.57 mmol, 75%) was obtained as a clear oil. $^1$H−NMR (300 MHz, $CDCl_3$) δ 8.28 (bs, 1H, OH), 5.11 (bd, *J* = 7.8 Hz, 1H, NH), 4.32−4.24 (m, 1H, CαH), 2.19−2.12 (m, 2H, C≡C$CH_2$), 2.01−1.87 (m, 2H, $CH_2$), 1.75 (s, 3H, C≡CMe), 1.62−1.51 (m, 2H, $CH_2$), 1.43 (s, 9H, $CMe_3$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 176.1, 155.2, 80.1, 78.0, 76.1, 53.4, 31.6, 28.3, 24.9, 18.5, 3.5; IR ν 2928, 1712, 1511, 1394, 1367, 1163 cm$^{-1}$; HMRS (FAB$^+$) calcd for $C_{13}H_{22}O_4N$ 256.15488, found 256.1549.




**(2*R*)-2-[(*tert*-Butoxycarbonyl)amino]-5-hexynoic acid (33) .** Using the previously described protocol, $H_2$N-hMpg-OH **31** (150 mg, 1.06 mmol) was converted to Boc-hMpg-OH (212 mg, 0.88 mmol, 83%), a white solid. $[\alpha]_D$ = –10.7 (*c* = 1.13, $CH_2Cl_2$); $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.21−5.15 (bs, 1H, NH), 4.44−4.30 (m, 1H, CαH), 2.32−2.25 (m, 2H, C≡C$CH_2$), 2.14−2.03 (m, 1H, $CH_2$), 1.96−1.86 (m, 1H, $CH_2$), 1.78 (s, 3H, C≡CMe), 1.47 (s, 9H, $CMe_3$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 176.5, 155.3, 81.6, 80.2, 77.0, 52.9, 31.4, 28.3, 15.3, 3.6; IR ν 2975, 1712, 1511, 1247, 1161 cm$^{-1}$; HMRS (FAB$^+$) calcd for $C_{12}H_{20}O_4N$ 242.13923, found 242.1392.

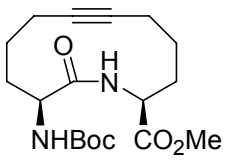



**2-[(*tert*-Butoxycarbonyl)amino]isobutyric acid (Boc-Aib-OH, 42).**[38] To a solution of $H_2$N-Aib-OH (2-aminoisobutyric acid, 3.35 g, 32.5 mmol) in aqueous 2 M NaOH (16 mL) was added a solution of $Et_3N$ (4.5 mL, 32.5 mmol) and $Boc_2O$ (5.52 g, 25.3 mmol) in 1,4-dioxane (35 mL). The resulting mixture was stirred for 40 h. The dioxane was removed *in vacuo* and the aqueous solution was acidified to pH = 2 using 2 M HCl. The mixture was extracted using EtOAc (3 × 30 mL) and the organic layer were washed (brine), dried ($MgSO_4$) and evaporation yielded the desired compound **42** (3.33 g, 16.4 mmol) as a white solid in 65%. $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.05 (bs, 1H, NH), 1.53 (s, 6H, 2 × Me), 1.45 (s, 9H, Boc); $^{13}$C−NMR (100 MHz, $CDCl_3$) δ 179.5, 155.0, 80.0, 56.1, 28.3, 25.4 (2 ×).

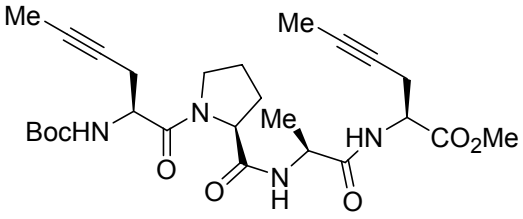



**Boc-hhMpg-hhMpg-OMe (28).** To a solution of Boc-hhMpg-OH (616 mg, 2.30 mmol) and $Et_3N$ (0.36 mL, 2.56 mmol) in $Et_2O$ at 0 °C was added dropwise isobutyl chloroformate (0.3 mL, 2.31 mmol). After warming to room temperature and stirring for 30 min the reaction mixture was filtered over a glass filter under a nitrogen stream. DiPEA (1.18 mL, 6.91 mmol) and (*S*)-2-amino-oct-6-ynoic acid methyl ester (475 mg, 2.31 mmol) were added and the reaction mixture was allowed to stir overnight. The mixture was evaporated *in vacuo* and purified using flash chromatography (40% EtOAc in heptane), yielding **28** as a white solid (814 mg, 2.00 mmol, 87%). $[\alpha]_D$ = +14.2 ($Hg_{385}$, *c* = 1.0, $CH_2Cl_2$); $^1$H–NMR (400 MHz, $CDCl_3$) δ 6.61 (d, *J* = 7.4 Hz, 1H, NH), 5.06 (d, *J* = 8.0 Hz, 1H, NHBoc), 4.83–4.78 (m, 1H, C*H*NHBoc), 4.09–3.96 (m, 1H, C*H*$CO_2$Me), 3.72 (s, 3H, OMe) 2.34–2.20 (m, 4H, 2 × C≡C$CH_2$), 1.95–1.82 (m, 2H, $CH_2$), 1.77–1.62 (m, 8H, 2 × C≡C*Me*, $CH_2$),), 1.58-1.45 (m, 4H, 2 × $CH_2$), 1.42 (s, 9H, Boc); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 172.4, 171.7, 155.6, 80.0, 78.3, 78.0, 77.4, 76.2, 54.1, 52.3, 71.8, 31.8, 31.5, 28.2, 24.8, 24.7, 18.4, 18.3, 3.4 (2 ×); $^{15}$N–NMR (40.5 MHz, $CDCl_3$) δ 123.7 (NHBoc), 115.4 (NH); IR ν 2922, 2865, 2240, 1746, 1660, 1531, 1367, 1170 cm$^{-1}$; HRMS (EI): calculated for $C_{22}H_{34}N_2O_5$ 406.2468, found 406.2448.

**11-*tert*-Butoxycarbonylamino-12-oxo-azacyclododec-6-yne-2-carboxylic acid methyl ester (24).** To a stirred solution of Boc-hhMpg-hhMpg-OMe (33 mg, 81 µmol) and ($^t$BuO)$_3$W≡C$^t$Bu (5.5 mg, 6.9 µmol) was added toluene (5 mL) and the reaction was heated to 80 °C for 1.5 h. After cooling down and removal of the volatiles, purification using flash chromatography resulted in **24** as an off-white solid (22 mg, 62 µmol, 77%). $[\alpha]_D$ = –14.6 (*c* = 1.0, $CH_2Cl_2$); $^1$H–NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.7 Hz, 1H, NH), 6.08 (d, *J* = 8.3 Hz, 1H, NHBoc), 4.78 (q, *J* = 6.8 Hz, 1H, CαH) 4.27 (q, *J* = 7.9 Hz, 1H, CαH), 3.73 (s, 3H, OMe), 2.17–2.15 (m, 4H, 2 × C≡C$CH_2$), 2.07–1.96 (m, 2H, $CH_2$), 1.79–1.52 (m, 4H, 2 × CαC$H_2$), 1.45 (s, 9H, Boc), 0.89–0.83 (m, 2H, $CH_2$); $^{13}$C–NMR (100 MHz, $CDCl_3$) δ 173.2, 171.8, 155.8, 80.4, 80.2, 79.3, 53.8, 52.5, 51.2, 32.8 (2 ×), 28.1, 24.6, 24.2, 18.3 (2 ×); IR ν 3313, 2931, 2865, 2249, 1744, 1667, 1520, 1366, 1170 cm$^{-1}$; HRMS (EI): calculated for $C_{18}H_{28}N_2O_5$ 352.1998, found 352.1984.



*Representative procedure for the synthesis of the tetramers.*

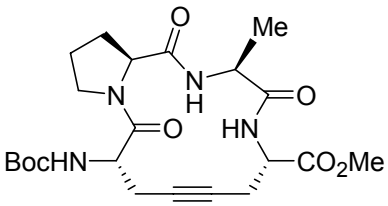



**Boc-Mpg-Pro-Ala-Mpg-OMe (45).** To a stirred suspension of (*S*)-2-amino-hex-4-ynoic acid (**30**, 300 mg, 2.36 mmol) in MeOH (15 mL) was slowly added $SOCl_2$ (500 µL, 6.72 mmol) and the resulting clear solution was refluxed for 2 h. After cooling to ambient temperature, the solvents were removed *in vacuo*. The crude methyl ester was resuspended in $CH_2Cl_2$ (15 mL) and DiPEA (1.0 mL, 5.9 mmol), PyBOP (1.43 g, 2.75 mmol) and Boc-Ala-OH (531 mg, 2.80 mmol) were added. After stirring for 16 h, the product was evaporated and purified using flash chromatography (50% EtOAc in heptane) yielding **43** as a fluffy white solid (432 mg, 1.36 mmol, 59%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 6.84 (bd, *J* = 7.9 Hz, 1h, CONH), 5.16 (bd, *J* = 7.3 Hz, 1H, NHBoc), 4.69−4.59 (m, 1H, CαH), 4.30−4.13 (m, 1H, CαH), 3.74 (s, 3H, OMe), 2.76−2.60 (m, 2H, C≡C$CH_2$), 1.72 (t, *J* = 2.5 Hz, 3H, C≡CMe), 1.42 (s, 9H, Boc), 1.35 (d, *J* = 7.3 Hz, Me). The solid was dissolved in 2 M HCl EtOAc (20 mL) and stirred for 30 min. After complete conversion (based on TLC), the solvents were stripped *in vacuo* and the HCl-salt was redissolved in $CH_2Cl_2$ (30 mL) and DiPEA (611 µL, 3.60 mmol), PyBOP (720 mg, 1.38 mmol) and Boc-Pro-OH (300 mg, 1.39 mmol) were added. The resulting solution was stirred overnight at ambient temperature. After evaporation and further purification, the desired tripeptide **44** was obtained (352 mg, 0.86 mmol, 62%) as a colorless oil. $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.04 (bs, 1H, NH), 6.85 (bs, 1H, NH), 4.60−4.50 (m, 1H, CαH), 4.50−4.32 (m, 1H, CαH), 4.29−4.10 (m, 1H, CαH), 3.70 (s, 3H, OMe), 3.47−3.23 (m, 2H, $CH_2N$), 2.68−2.53 (m, C≡C$CH_2$), 2.23−1.75 (m, 4H, 2 × $CH_2$), 1.68 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.39 (s, 9H, Boc), 1.33 (d, *J* = 6.9 Hz, 3H, MeCαH). The previously made tripeptide **44** (500 mg, 1.22 mmol) was dissolved in 2 M HCl EtOAc (20 mL) and stirred for 30 min. After complete conversion (based on TLC) the solvents were stripped *in vacuo* and the HCl-salt was redissolved in $CH_2Cl_2$ (30 mL). DiPEA (550 µL, 3.24 mmol) and **32** (309 mg, 1.35 mmol) were added and after 5 min, followed by PyBOP (700 mg, 1.35 mmol). After stirring overnight at room temperature, the volatiles were removed and the mixture was further purified using flash chromatography (100% EtOAc) yielding the desired tetramer **45** (550 mg, 1.02 mmol, 76%) as white solid. $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.13 (bd, *J* = 7.3 Hz, 1H, NH-Mpg-OMe), 7.08 (bd, *J* = 7.5 Hz, 1H, NH-Ala), 5.35 (bd, *J* = 8.0 Hz, 1H, NHBoc), 4.62−4.54 (m, 3H, 3 × CαH), 4.47−4.40 (m, 1H, CαH-Ala), 3.81−3.78 (m, 1H, $CH_2N$), 3.72 (s, 3H, OMe), 3.72−3.66 (m, 1H, $CH_2N$), 2.67−2.63 (m, 2H,

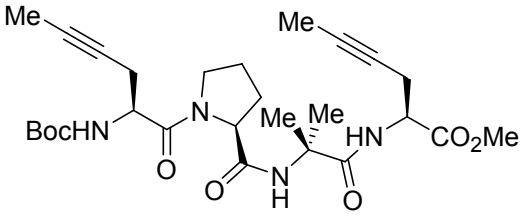
C≡CCH$_2$), 2.57–2.55 (m, 2H, C≡CCH$_2$), 2.25 (m, 1H, CH$_2$), 2.06–1.97 (m, 3H, CH$_2$), 1.72 (t, *J* = 2.4 Hz, 6H, 2 × C≡CMe), 1.37 (s, 9H, Boc), 1.33 (d, *J* = 5.8 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, CDCl$_3$) δ 173.3, 173.1, 172.4, 172.0, 156.4, 81.5, 80.4, 80.2, 75.3, 74.8, 74.5, 61.7, 53.9, 52.7, 52.3, 50.4, 49.4, 29.6, 29.2, 26.5, 25.0, 23.7, 18.8, 4.94, 4.88. IR v 3293, 2926, 2362, 2341, 1742, 1642, 1520, 1450, 1369, 1245, 1168, 1048, 847 cm$^{-1}$; HRMS (FAB) m/z calcd for $C_{26}H_{48}N_4O_7$: 519.2819, found 519.2824 (M + H)$^+$.

*General procedure for ring-closing alkyne metathesis of the tetramers.*

**Methyl (3*S*, 8*S*, 11*S*, 14*S*)-3-[(*tert*-butoxycarbonyl)amino]-11-methyl-2,10,13-trioxo-1.9.12-triazabicyclo[12.3.0]heptadec-5-yne-8-carboxylate**

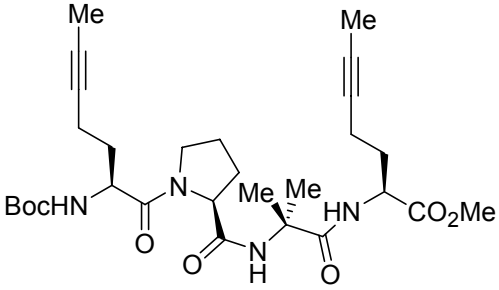



**(46).** The reaction was performed under an Ar atmosphere in flame-dried glassware. To a solution of ($^t$BuO)$_3$W≡C$^t$Bu (**1**, 10 mg, 0.02 mmol) in dry C$_6$H$_5$Cl (5.5 mL) was added a solution of **45** (200 mg, 0.39 mmol) in dry C$_6$H$_5$Cl (30 mL) and the reaction mixture was stirred at 80 °C for 3 h. The solvent was concentrated and the crude mixture was purified by column chromatography (EtOAc) to give **46** as a crystaline solid (112 mg, 0.23 mmol) in 62% yield. $^1$H–NMR (400 MHz, CDCl$_3$) δ 7.63 (bd, 1H, NH-Mpg), 6.58 (bd, 1H, NH-Ala), 5.80 (bd, 1H, NHBoc), 4.81 (m, 1H, CαHCO$_2$Me), 4.60 (m, 1H, CαHNHBoc), 4.31 (m, 1H, CαH-Pro), 4.17 (m, 1H, CαH-Ala), 3.82 (s, 3H, OMe), 3.75–3.62 (m, 1H, NCH$_2$), 3.61–3.56 (m, 1H, NCH$_2$), 2.71–2.62 (m, 4H, 2 × C≡CC*H*$_2$), 2.24–1.90 (m, 4H, 2 × CH$_2$), 1.53 (d, 3H, *J* = 7.1 Hz, Me-Ala), 1.44 (s, 9H, Boc); $^{13}$C–NMR (75 MHz, CDCl$_3$) δ 170.5, 170.3, 170.1, 169.6, 154.3, 79.6, 78.2, 76.8, 61.7, 52.4, 50.9, 50.6, 50.2, 47.3, 29.5, 28.4, 28.1, 25.5, 22.6, 22.5; IR v 3311, 2926, 1745, 1643, 1504, 1444, 13678, 1163, 1061, 1030, 911, 734 cm$^{-1}$; HRMS (FAB) *m/z* calcd for $C_{22}H_{32}N_4O_7Na$: 487.21687, found 487.21656 (M + Na)$^+$.



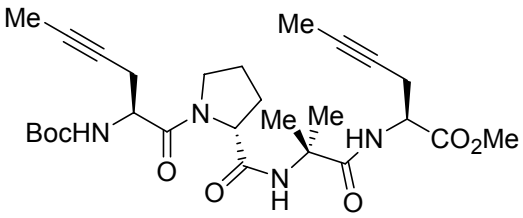

**Boc-Mpg-Pro-Aib-Mpg-OMe (38).** Following the example for tetramer synthesis, (*S*)-2-amino-hex-4-ynoic acid methyl ester (100 mg, 0.56 mmol) was coupled with Boc-Aib-OH (191 mg, 0.94 mmol) yielding yellowish oil **34** (155 mg, 0.49 mmol, 88%). $^1$H–NMR (400 MHz, CHCl$_3$) δ 7.13–7.04 (m, 1H, NH), 5.04 (s, 1H, NHBoc), 4.65–4.58 (m, 1H, CαH), 3.71 (s, 3H, OMe), 2.72–2.56 (m, 2H, C≡CCH$_2$), 1.70 (t, *J* = 2.4 Hz, 3H, C≡CMe),

1.46 (s, 3H, Me-Aib), 1.45 (s, 3H, Me-Aib), 1.40 (s, 9H, Boc);$^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.3, 171.1, 154.4, 80.1, 78.9, 72.9, 56.6, 52.4, 51.0, 18.1, 25.3, 25.0, 22.5, 3.4; IR ν 3311.2, 2977.6, 2935.1, 1783.8, 1714.4, 1666.2, 1506.1, 1160.9 cm$^{-1}$; MS (EI) m/z: 326, 253, 158, 102, 88, 82, 58. The deprotected dipeptide (75 mg, 0.33 mmol) was reacted with Boc-Pro in similar fashion, yielding **36** as a white solid (81 mg, 0.19 mmol, 58%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.40−7.27 (m, 1H, NH), 6.80 (bs, 1H, NH), 4.54−4.48 (m, 1H, CαH-Mpg), 4.15−4.06 (m, 1H, CαH-Pro), 3.66 (s, 3H, OMe), 3.48−3.35 (m, 2H, $CH_2N$), 2.72−2.56 (m, 2H, C≡C$CH_2$), 2.20−2.00 (m, 3H, $CH_2$), 2.00−1.89 (m, 1H, $CH_2$), 1.70 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.47 (s, 3H, Me-Aib), 1.44 (s, 3H, Me-Aib), 1.40 (s, 9H, Boc). After deprotection of the tripeptide (147 mg, 0.34 mmol), **32** (106 mg, 0.47 mmol) was coupled which resulted in **38** as a white solid (134 mg, 0.25 mmol, 74%). $^1$H−NMR (400 MHz, $CHCl_3$) δ 7.31−7.26 (m, 1H, NH), 7.16 (bd, *J* = 7.8 Hz, 1H, NH), 5.66 (bd, *J* = 7.3 Hz, NH), 4.62 (m, 1H, CαH), 4.48 (m, 1H, CαH), 4.41 (m, 1H, CαH), 3.75 (s, 3H, OMe), 3.80−3.70 (m, 2H, $CH_2N$), 2.66 (m, 2H, C≡C$CH_2$), 2.54 (m, 2H, C≡C$CH_2$), 2.20−2.00 (m, 3H, $CH_2$), 2.00−1.89 (m, 1H, $CH_2$), 1.76 (s, 3H, C≡CMe), 1.76 (s, 3H, C≡CMe), 1.53 (s, 3H, Me-Aib), 1.49 (s, 3H, Me-Aib), 1.46 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.1, 174.0, 173.2, 171.5, 155.0, 80.0, 79.0, 78.6, 73.7, 73.4, 60.7, 57.2, 52.4, 51.4, 50.8, 47.9, 40.0, 28.2, 27.6, 25.6, 25.7, 25.0, 24.7, 23.4, 22.2, 3.1, 3.0; IR ν 3313.7, 2978.8, 1742.5, 1639.8, 1520.0, 1441.4, 1366.7, 1247.5, 1167.7, 1051.4, 915.1, 846.1, 733.1 cm$^{-1}$; HRMS (SIMS) m/z calcd for $C_{27}H_{40}N_4O_7$ 555.2795, found 555.2824 (M + Na)$^+$.

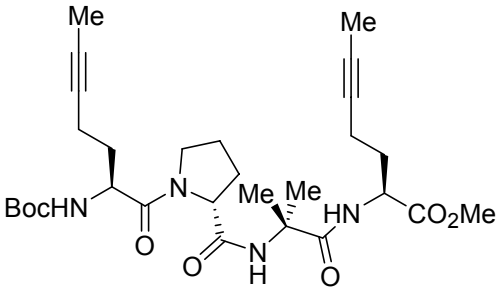



**Boc-hMpg-Pro-Aib-hMpg-OMe (39).** Following the general example of tetramer synthesis, $H_2N$-hMpg-OMe (200 mg, 1.04 mmol) was coupled with Boc-Aib-OH (250 mg, 1.23 mmol) resulting in **35** as a clean oil. The crude product was deprotected, condensed with Boc-Pro-OH (181 mg, 0.84 mmol) and isolated yielding **37** as a white solid (188 mg, 0.45 mmol, 43%, 3 steps). Deprotection and subsequent condensation with Boc-hMpg-OH (125 mg, 0.51 mmol) resulted in tetrapeptide **39** as a white solid (137 mg, 0.24 mmol, 54%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.21 (bd, *J* = 6.3 Hz, 1H, NH-hMpg), 6.79 (bs, 1H, NH-Aib), 5.38 (bd, *J* = 6.5 Hz, 1H, NHBoc), 4.68−4.49 (m, 2H, 2 × CαH), 4.41−4.29 (m, 1H, CαH), 3.70 (s, 3H, OMe), 3.78−3.69 (m, 2H, $CH_2N$), 2.27−1.83 (m, 12H, 6 × $CH_2$), 1.74 (t, *J* = 2.3 Hz, 6H, 2 × C≡CMe), 1.54 (s, 3H, Me-Aib), 1.44 (s, 3H, Me-Aib), 1.42 (s, 9H, Boc); $^{13}$C−NMR

(75 MHz, $CDCl_3$) δ 174.1, 172.6, 172.0, 171.0, 155.5, 80.3, 79.7, 77.8, 76.5, 76.4, 60.3, 57.2, 52.2, 51.8, 51.2, 47.4, 31.8, 30.9, 28.1, 26.4, 25.1, 20.9, 15.22, 15.15, 14.0, 3.3, 3.2; IR ν 3313.2, 2975.7, 1683.1, 1636.5, 1520.0, 1445.0, 1169.7, 1049.9, 846.0 $cm^{-1}$; MS (FAB): m/z (%): 583 (12), 561 (5), 461 (14), 265 (8), 154 (21), 149 (9), 140 (9), 137(12), 136 (16), 98 (21), 77 (11), 70 (100), 58 (25), 57 (56).



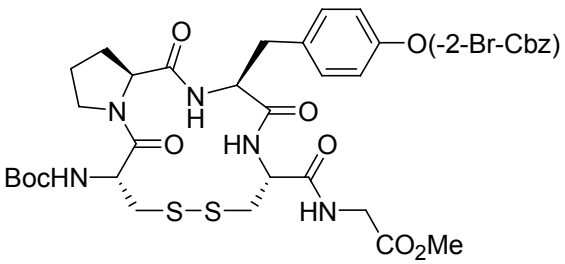

**Boc-Mpg-(*R*)-Pro-Aib-Mpg-OMe (40).** Using the standard peptide conditions previously described Boc-Aib-Mpg-OMe (**34**, 155 mg, 0.48 mmol) was deprotected and condensed with Boc-(*R*)-Pro-OH (127 mg, 0.59 mmol) resulting in the desired tripeptide (152 mg, 0.36 mmol, 76%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.40−7.27 (m, 1H, NH), 6.80 (bs, 1H, NH), 4.54−4.48 (m, 1H, CαH-Mpg), 4.15−4.06 (m, 1H, CαH-Pro), 3.66 (s, 3H, OMe), 3.48−3.35 (m, 2H, $CH_2N$), 2.72−2.56 (m, 2H, $C{\equiv}CCH_2$), 2.20−2.00 (m, 3H, $CH_2$), 2.00−1.89 (m, 1H, $CH_2$), 1.70 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.47 (s, 3H, Me-Aib), 1.44 (s, 3H, Me-Aib), 1.40 (s, 9H, Boc); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 174.2, 171.9, 171.2, 155.4, 80.4, 78.4, 73.0, 60.7, 60.4, 56.6, 52.4, 46.9, 28.0, 25.2, 24.5, 24.3, 21.8, 15.0, 3.2. The tripeptide (152 mg, 0.36 mmol) was deprotected and coupled to **32** (106 mg, 0.47 mmol) resulting in **40** as a white solid (134 mg, 0.25 mmol, 71%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.14 (bd, *J* = 8.0 Hz, 1H, NH-Mpg-OMe), 6.99 (s, 1H, NH-Aib), 5.65 (d, *J* = 7.6 Hz, 1H, NHBoc), 4.57 (q, *J* = 8.0 Hz, 1H, CαH), 4.56 (q, *J* = 7.3 Hz, 1H, CαH), 4.36−4.33 (m, 1H, CαH), 3.70 (s, 3H, OMe), 3.80−3.71 (m, 2H, $CH_2N$), 2.63−2.61 (m, 2H, $C{\equiv}CCH_2$), 2.52−2.49 (m, 2H, $C{\equiv}CCH_2$), 2.17−1.84 (m, 4H, 2 × $CH_2$), 1.72 (s, 6H, 2 × C≡CMe), 1.48 (s, 3H, Me-Aib), 1.45 (s, 3H, Me-Aib), 1.39 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.2, 171.7, 171.0, 170.7, 155.4, 80.0, 78.6, 78.2, 73.7, 73.3, 60.9, 57.0, 52.5, 51.6, 51.3, 47.6, 28.4, 26.1 (2 ×), 25.9, 24.2, 22.2, 15.1, 14.1, 3,4, 3.3; IR ν 3314, 2980, 1743, 1638, 1520, 1367, 1248, 1168 $cm^{-1}$; HRMS (FAB) m/z calcd for $C_{27}H_{40}N_4O_7$ 555.2795, found 555.2939 $(M + Na)^+$.




**Boc-hMpg-(*R*)-Pro-Aib-hMpg-OMe (41).** Using standard peptide conditions previously described $H_2N$-hMpg-OH (100 mg, 0.71 mmol) was coupled with Boc-Aib-OH (171 mg, 0.84 mmol) resulting in a white solid (150 mg, 0.46 mmol, 65%). $^1$H−NMR (300 MHz, $CDCl_3$) δ

7.05 (bd, $J$ = 6.6 Hz, 1H, NH), 4.97 (bs, 1H, NHBoc), 4.64−4.53 (m, 1H, CαH), 3.69 (s, 3H, OMe), 2.20−2.10 (m, 2H, C≡C$CH_2$), 2.07−1.78 (m, 2H, $CH_2$Cα), 1.72 (t, $J$ = 2.4 Hz, 3H, C≡CMe), 1.46 (s, 3H, Me-Aib), 1.44 (s, 3H, Me-Aib), 1.40 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.3, 172.3, 154.4, 80.1, 77.4, 76.4, 56.5, 52.1, 51.6, 31.2, 28.1, 25.7, 25.0, 14.7, 3.4; IR ν 3317, 2978, 2924, 2468, 1749, 1682, 1655, 1522, 1165 cm$^{-1}$. The dipeptide (150 mg, 0.46 mmol) was then deprotected and condensed with Boc-(*R*)-Pro (118 mg, 0.55 mmol) yielding a white solid (147 mg, 0.34 mmol, 70%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.43−7.30 (m, 1H, NH-hMpg), 6.80 (bs, 1H, NH-Aib), 4.56−4.46 (m, 1H, CαH-hMpg), 4.13−4.08 (m, 1H, CαH-Pro), 3.70 (s, 3H, OMe), 3.50−3.27 (m, 2H, $CH_2$N), 2.20−1.80 (m, 8H, 4 × $CH_2$), 1.70 (t, $J$ = 2.4 Hz, 3H, C≡CMe), 1.49 (s, 3H, Me-Aib), 1.43 (s, 3H, Me-Aib), 1.42 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.4, 172.6, 171.9, 155.4, 80.5, 77.2, 76.2, 60.9, 56.9, 52.2, 51.9, 47.2, 30.7, 29.2, 28.1, 25.6, 24.9, 24.6, 15.2, 3.4. The tripeptide (147 mg, 0.34 mmol) was deprotected and coupled with Boc-hMpg-OH (104 mg, 0.43 mmol) yielding the desired tetrapeptide **41** (150 mg, 0.27 mmol, 79%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.18 (bd, $J$ = 7.6 Hz, 1H, NH-hMpg), 6.80 (bs, 1H, NH-Aib), 5.46 (bd, $J$ = 6.5 Hz, 1H, NHBoc), 4.63−4.46 (m , 2H, 2 × CαH), 4.35−4.14 (m, 1H, CαH), 3.71 (s, 3H, OMe), 3.78−3.69 (m, 2H, $CH_2$N), 2.27−1.83 (m, 12H, 6 × $CH_2$), 1.74 (t, $J$ = 2.3 Hz, 6 H, × C≡CMe), 1.54 (s, 3H, Me-Aib), 1.44 (s, 3H, Me-Aib), 1.43 (s, 9H, Boc); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.6, 172.9, 171.6, 171.1, 155.6, 79.9, 77.6, 77.4, 76.7, 76.2, 60.9, 57.0, 52.3, 51.4, 47.1, 31.4, 31.2, 28.4, 28.3, 25.8, 24.8, 24.6, 15.2, 3.3 (2 ×); IR ν 3297.7, 2975.4, 1637.7, 1524.0, 1444.3, 1366.0, 1246.4, 1168.3, 1047.1 cm$^{-1}$; HRMS (FAB) m/z calcd for $C_{29}H_{45}N_4O_7$ 561.3288, found 561.3376 $(M + H)^+$.

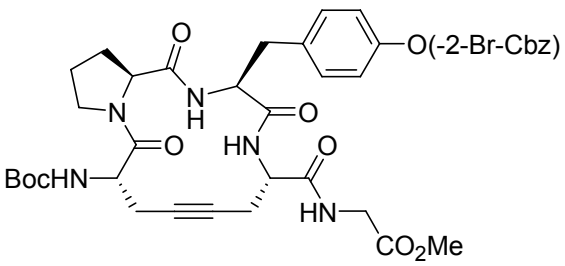
**Methyl (3*S*, 8*S*, 11*S*, 14*S*)-3-[(*tert*-butoxycarbonyl)amino]- 11-[4-([(2-bromobenzyl)oxy]carbonyloxy)benzyl]-[(2,10,13-trioxo-1,9,12-triaza-5,6-dithiabicyclo[12.3.0]heptadecane-8-ylcarbonyl)amino]acetate (55).**



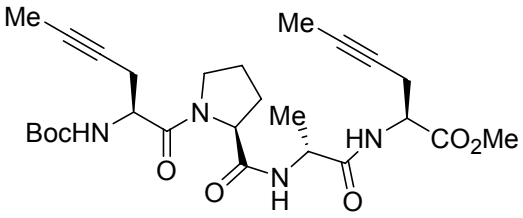

A solution of the suitably protected pentapeptide (73 mg, 0.072 mmol) in DMF (7.2 mL) was poured, in one portion, into a well-stirred solution of iodine (220 mg, 0.86 mmol) in MeOH (65 mL). After 5 min, a solution of ascorbic acid (166 mg, 0.94 mmol) in citrate buffer (pH = 5, 14.4 mL) was added, producing within about 15 s a colorless and slightly turbid solution. The solution was concentrated to ≈ 10 mL and then extracted with $CH_2Cl_2$, dried ($MgSO_4$), and evaporated. The

crude was purified by column chromatography (66% to 100% EtOAc in petroleum ether) to give **55** (50 mg, 80%). $[\alpha]_D$ = +42.3 (*c* = 0.14, $CH_2Cl_2$); $^1$H−NMR δ 7.62 (d, *J* = 7.9 Hz, 1H, Ar), 7.53 (d, *J* = 7.3 Hz, 1H, Ar), 7.53 (t, *J* = 7.4 Hz, 1H, Ar) 7.26 (t, *J* = 7.0 Hz, 1H, Ar), 7.15 (m, 6H, Ar, NH-Gly, NH-Cys), 6.11 (d, *J* = 9.5 Hz, 1H, NH-Tyr), 5.45 (d, *J* = 7.8 Hz, 1H, NH-Cys), 5.36 (q, *J* = 12.8 Hz, *J* = 17.4 Hz, 2H, $CH_2Ph$), 5.02 (bs, 1H, CαH-Tyr), 4.75 (t, *J* = 8.9 Hz, 1H, CαH-Cys), 4.59 (m, 1H, CαH-Cys), 4.42 (m, 1H, CαH-Pro), 4.04 (dd, *J* = 5.6 Hz, *J* = 18.1 Hz, 1H, CαH-Gly), 3.98 (dd, *J* = 5.4 Hz, *J* = 18.2 Hz, 1H, CαH-Gly), 3.76 (s, 3H, OMe), 3.71 (m, 1H, $CH_2N$), 3.50 (dd, *J* = 5.5 Hz, *J* = 14.0 Hz, 1H, αH-Tyr), 3.44 (m, 1H, $CH_2N$), 3.30 (q, *J* = 11.5 Hz, *J* = 15.7 Hz, 1H, $CH_2S$), 3.14 (m, 2H, 2 × $CH_2S$), 3.06 (m, 2H, αH Tyr, $CH_2S$), 2.28 (m, 1H, $CH_2$), 2.05 (m, 1H, $CH_2$), 1.93 (m, 2H, $CH_2$), 1.42 (s, 9H, Boc); $^{13}$C−NMR δ 170.8, 170.2, 169.7, 150.36, 134.0, 132.8, 130.1, 129.9, 127.5, 120.9 (Ar), 80.7, 69.5, 62.1, 57.5, 52.2, 52.0, 51.3, 47.5, 41.1, 40.1, 35.3, 33.8, 29.3, 28.1, 24.9; HRMS *m/z* calcd for $C_{36}H_{45}BrN_5O_{11}S_2$ 866.1740, found 866.1692 $(M + H)^+$.

**Methyl (3*S*, 8*S*, 11*S*, 14*S*)-3-[(*tert*-butoxycarbonyl)amino]- 11-[4-([(2-bromobenzyl)oxy]carbonyloxy)benzyl]-[(2,10,13-trioxo-1,9,12-triaza-bicyclo[12.3.0]heptadec-5-yn-8-ylcarbonyl)amino]acetate (54).**



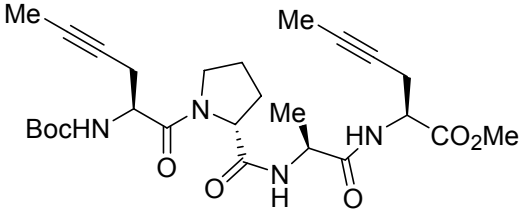

Using the general procedure for ring closing alkyne metathesis peptide **53** (200 mg, 0.23 mmol) was reacted upon which **54** was obtained (68 mg, 0.08 mmol, 36%). $[\alpha]_D$ = –2.6 (*c* = 0.3, $CH_2Cl_2$); $^1$H−NMR δ 8.15 (bs, 1H, NH-Gly), 7.89 (d, *J* = 7.9 Hz, 1H, NH-Mpg), 7.61−7.06 (m, 8H, Ar), 6.54 (d, *J* = 7.0 Hz, 1H, NH-Tyr), 5.98 (d, *J* = 8.3 Hz, 1H, NH Boc), 5.35 (s, 2H, $CH_2Ph$), 4.63 (bs, 1H, CαH-Mpg), 4.55 (bs, 1H, CαH-Mpg), 4.29 (bs, 1H, CαH-Tyr), 4.16 (t, *J* = 7.5 Hz, 1H, CαH-Pro), 4.10 (m, 1H, CαH-Gly), 3.91 (m, 1H, CαH Gly), 3.74 (s, 3H, OMe), 3.72−3.53 (m, 3H, 2 × $CH_2N$, $CH_2Ar$), 3.32 (m, 1H, $CH_2Ar$), 2.73−2.59 (m, 4H, 2 × C≡C$CH_2$), 2.05 (m, 2H, $CH_2$), 1.95 (m, 1H, $CH_2$), 1.84 (m, 1H, $CH_2$), 1.40 (s, 9H, Boc); $^{13}$C−NMR δ 172.3, 171.6, 170.9, 170.7, 170.4, 155.4, 155.3, 135.2, 132.8, 129.9, 127.5, 123.3, 121.5, 121.0, 80.0, 78.8, 77.2, 69.5, 62.0, 56.8, 52.1, 51.7, 50.5, 47.1, 41.1, 34.6, 28.6, 28.1, 25.5, 22.8, 21.7; HRMS *m/z*: calcd for $C_{38}H_{45}BrN_5O_{11}$: 826.2298, found 826.2257 $(M + H)^+$.



**Boc-Mpg-Pro-(*R*)-Ala-Mpg-OMe (49).** Using the general example of tetramersynthesis (*S*)-2-amino-hex-4-ynoic acid (**30**, 350 mg, 2.75 mmol) was condensed with Boc-(*R*)-Ala-OH (625 mg, 3.30 mmol) yielding **47** as a clean oil (438 mg, 1.44 mmol, 51%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 6.88 (bd, *J* = 8.0 Hz, 1H, NH), 5.09−4.98 (m, 1H, NHBoc), 4.71−4.62 (m, 1H, CαH-Mpg), 4.32−4.16 (m, 1H, CαH-Ala), 3.76 (s, 3H, OMe), 2.74−2.65 (m, 2H, C≡C$CH_2$), 1.75 (t, *J* = 2.5 Hz, 3H, C≡CMe), 1.76 (s, 9H, Boc), 1.37 (d, *J* = 7.1 Hz, 3H, Me-Ala); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 171.5, 154.9, 79.9, 79.1, 74.1, 72.7, 60.3, 52.4, 51.1, 28.1, 24.7, 23.4, 3.3. After deprotection and subsequent condensation with Boc-Pro-OH (362 mg, 1.68 mmol), **48** was recovered as a white solid (416 mg, 1.01 mmol, 72%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.09−6.48 (m, 2H, 2 × NH), 4.66−4.50 (m, 2H, 2 × CαH), 4.30−4.20 (m, 1H, CαH), 3.74 (s, 3H, OMe), 3.53−3.31 (m, 2H, $CH_2$N), 2.71−2.63 (m, 2H, C≡C$CH_2$), 2.24−1.80 (m, 4H, 2 × $CH_2$), 1.74 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.44 (s, 9H, Boc), 1.38 (d, *J* = 6.9 Hz, 3H, Me-Ala). After deprotection the tripeptide (104 mg, 0.30 mmol) was condensed with **32** (75.2 mg, 0.33 mmol) providing **49** as a white foamy solid (88 mg, 0.17 mmol, 57%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.15 (bd, *J* = 7.6 Hz, 1H, NH-Mpg), 6.96 (bd, *J* = 7.6 Hz, 1H, NH-Ala), 5.35 (bd, *J* = 8.4 Hz, NHBoc), 4.59−4.50 (m, 4H, 4 x αH), 3.89 (m, 1H, $CH_2$N), 3.73 (s, 3H, OMe), 3.71 (m, 1H, $CH_2$N), 2.64−2.48 (m, 4H, C≡C$CH_2$), 2.30−1.99 (m, 4H, 2 × $CH_2$), 1.75 (s, 3H, C≡CMe), 1.74 (s, 3H, C≡CMe), 1.41 (s, 9H, Boc), 1.35 (d, *J* = 7.2 Hz, 3H, Me); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 171.1, 170.8, 170.4, 168.9, 154.5, 79.7, 79.2, 79.0, 73.8, 72.5, 60.3, 52.2, 51.0, 50.7, 48.2, 47.8, 28.124.7, 24.6, 23.5, 22.1, 17.9, 16.2, 3.4, 3.3; IR v 3317.2, 2976.3, 1744.8, 1641.8, 1525.9, 1443.6, 1367.2, 1248.8, 1166.9, 782.3 $cm^{-1}$; HRMS (FAB) m/z calcd for $C_{26}H_{48}N_4O_7$ $(M + H)^+$ 519.2819, found 519.2824.

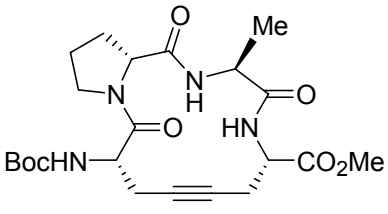
**Methyl (3*S*, 8*S*, 11*S*, 14*R*)-3-[(*tert*-butoxycarbonyl)amino]-11-methyl-2,10,13-trioxo-1.9.12-triazabicyclo[12.3.0]heptadec-5-yne-8-carboxylate (50).** Following the general example for cyclization, Boc-Mpg-Pro-(*R*)-Ala-Mpg-OMe (**49**, 81 mg, 0.16 mmol) was reacted, resulting in a recovery of **50** as a crystalline solid (51 mg, 0.11 mmol, 71%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.92 (bd, *J* = 8.2 Hz, 1H, NH), 6.59 (bd, *J* = 8.3 Hz, 1H, NH), 5.89 (bd, *J* = 8.3 Hz, 1H, NHBoc), 4.87 (m, 1H, CαH), 4.62 (m, 1H, CαH), 4.45 (t, *J* = 7.6 Hz, 1H, CαH), 4.33 (t, *J* = 7.1 Hz, 1H,

CαH), ,3.83 (s, 3H, OMe), 3.65 (m, 1H, $CH_2N$), 3.55 (m, 1H, $CH_2N$), 2.72−2.56 (m, 4H, C≡$CCH_2$), 2.33, 2.05 (m, 4H),1.47 (d, *J* = 7.6 Hz, 3H), 1.44 (s, 9H, Boc); $^{13}$C−NMR (75.5 MHz) δ 171.2, 170.5 (2 ×), 169.8, 154.5, 79.7, 78.3, 77.2, 61.3, 52.6, 50.7 (2 ×), 50.3, 47.5, 28.4, 27.8, 26.0, 23.4, 23.0, 18.1; IR ν 3311.9, 2974.5, 1744.0, 1639.3, 1510.6, 1445.4, 1165.9 cm$^{-1}$.




**Boc-Mpg-(*R*)-Pro-Ala-Mpg-OMe (51).** Following the general example for tetrapeptidesynthesis, Boc-Ala-Mpg-OMe (**43**, 89 mg, 0.28 mmol) was deprotected and condensed with Boc-(*R*)-Pro-OH (68 mg, 0.30 mmol). Upon isolation the tripeptide (80 mg, 0.20 mmol, 69%) was obtained. After deprotection the tripeptide was coupled with **32** (50 mg, 0.22 mmol) resulting in the desired tetrapeptide **51** (95 mg, 0.18 mmol, 92%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.20 (bd, *J* = 7.3 Hz, 1H, NH), 7.12 (bd, *J* = 6.8 Hz, 1H, NH), 5.68 (bd, *J* = 6.8 Hz, 1H, NH), 4.66−4.59 (m, 1H, CαH), 4.51−4.35 (m, 3H, 3 × CαH), 3.75 (s, 3H, OMe), 3.76−3.70 (m, 2H, $CH_2N$), 2.71−2.63 (m, 2H, C≡$CCH_2$), 2.55−2.48 (m, 2H, C≡$CCH_2$), 2.22−2.06 (m, 3H, 2 × $CH_2$), 2.01−1.93 (m, 1H, $CH_2$), 1.76 (s, 3H, C≡CMe), 1.75 (s, 3H, C≡CMe), 1.43 (s, 9H, Boc), 1.45−1.41 (m, 3H, Me); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 171.9, 171.3, 170.9, 169.9, 155.0, 78.8, 78.8, 78.0, 73.7, 73.0, 65.6, 65.5, 52.5, 51.2, 49.4, 47.5, 28.3, 25.0, 22.5, 22.4, 17.4, 15.3, 3.5 (2 ×); IR ν 3293.6, 2926.0, 1741.9, 1641.9, 1520.1, 1449.5, 1369.4, 1245.2, 1167.7, 846.7 cm$^{-1}$; (FAB) *m/z* :541, 519, 419, 378, 307, 154, 136.

**Methyl (3*S*, 8*S*, 11*R*, 14*S*)-3-[(*tert*-butoxycarbonyl)amino]-11-methyl-2,10,13-trioxo-1.9.12-triazabicyclo[12.3.0]heptadec-5-yne-8-carboxylate (52).** Following the general procedure for RCAM tetrapeptide **51** (65 mg, 0.12 mmol) was cyclized which upon isolation resulted in **52** as a white solid (33 mg, 0.07 mmol, 57%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.26 (bd, *J* = 8.0 Hz, 1H, NH), 6.81 (bd, *J* = 9.4 Hz, 1H, NH), 6.31 (bd, *J* = 10.3 Hz, 1H, NHBoc), 4.89−4.83 (m, 1H, CαH), 4.70−4.55 (m, 2H, 2 × CαH), 4.16−4.09 (m, 1H, CαH), 3.80 (s, 3H, OMe), 3.67−3.56 (m, 2H, $CH_2N$), 2.88−2.75 (m, 1H, C≡$CCH_2$), 2.71−2.52 (m, 3H, C≡$CCH_2$), 2.38−2.28 (m, 1H, $CH_2$), 2.22−2.12 (m, 1H, $CH_2$), 2.03−1.83 (m, 2H, $CH_2$), 1.46 (s, 9H, Boc), 1.45−1.42 (m, 3H, Me).

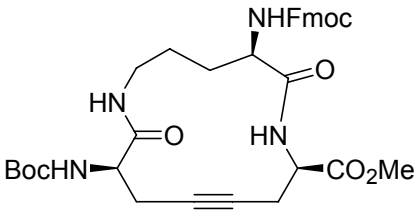


**Methyl (1*S*, 4*S*, 9*S*, 13*R*, 16*S*)-9-[(*tert*-butoxycarbonyl)amino]-16-(*tert*-butyl-dimethyl-silanyloxy)-[(2,10-dioxo-17-oxa-3,11-diaza-bicyclo[11.3.1]heptadec-6-yn-4-ylcarbonyl)amino]acetate (57).** Compound **57** was prepared according to the general metathesis protocol yielding **57** in 25%. $[\alpha]_D$ = –3.1 (*c* = 0.1, $CH_2Cl_2$). $^1$H–NMR δ 7.51(d, 1H, NH-Mpg-OMe), 7.49 (bs, 1H, NH-Gly), 6.82 (bs, 1H, NHSAA), 6.21 (d, 1H, NHBoc), 4.60 (m, 1H, CαH-Mpg-OMe), 4.45 (m, 1H, CαH-Mpg), 4.12 (m, 1H, CαH-Gly), 3.98 (m, 1H, CαH-Gly), 3.78 (m, 2H, $CH_2N$), 3.76 (s, 3H, OMe), 3.63 (m, 2H, 2 × CαH-SAA), 3.38 (m, 1H, C*H*OTBS), 2.92 (m, 1H, C≡C$CH_2$), 2.81 (m, 1H, C≡C$CH_2$), 2.60 (m, 1H, C≡C$CH_2$), 1.50 (s, 9H, Boc), 0.87 (s, 9H, SiC$Me_3$) 0.04 (s, 3H, SiMe), 0.02 (s, 3H, SiMe); $^{13}$C–NMR δ 169.8, 79.5, 75.2, 69.1, 52.9, 52.5, 43.0, 41.5, 29.7, 28.3, 26.6, 25.7, 23.0 (2 ×), –4.8, –4.9; HRMS *m*/*z*: calcd for $C_{29}H_{49}N_4O_9Si$ $(M + H)^+$: 625.3268, found 625.3220.

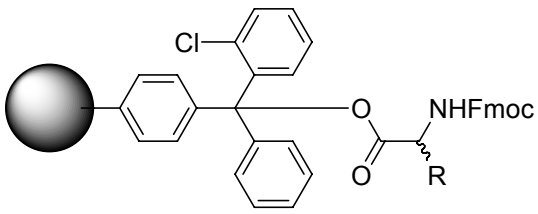
**Fmoc-Orn([(*R*)-2-amino-hex-4-ynoic acid]-Boc)-[(*R*)-2-amino-hex-4-ynoic acid]-OMe (61).** Following the general example for tetramer-synthesis (*S*)-**30** (135 mg, 0.76 mmol) was condensed with Fmoc-Orn(Boc)-OH (345 mg, 0.76 mmol) resulting in 60 as a white solid (490 mg, 0.85 mmol, inseparable from PyBOP residues). $^1$H–NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.60 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.39 (t, *J* = 7.3 Hz, 2H, Fmoc), 7.31 (t, *J* = 7.3 Hz, 2H, Fmoc), 7.20 (bs, 1H, NH), 6.77 (bs, 1H, NH), 5.59 (bs, 1H, NH), 4.69–4.62 (m, 2H, CαH, NH), 4.44–4.33 (m, 3H, $CH_2O$, CαH), 4.26–4.19 (m, 1H, CαHFmoc), 3.78 (s, 3H, OMe), 3.30–3.15 (m, 2H, $CH_2N$), 2.72–2.64 (m, 2H, C≡C$CH_2$), 1.81 (s, 3H, C≡CMe), 1.95–1.51 (m, 4H, 2 × $CH_2$), 1.44 (s, 9H, Boc). The crude dipeptide **60** was deprotected and upon condensation with **32** (144 mg, 0.66 mmol) **61** was obtained as a white solid (123 mg, 0.18 mmol, 24%, 3 steps). $^1$H–NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.60 (d, *J* = 7.3 Hz, 2H, Fmoc), 7.39 (t, *J* = 7.3 Hz, 2H, Fmoc), 7.31 (t, *J* = 7.3 Hz, 2H, Fmoc), 7.20 (bs, 1H, NH), 6.57 (bs, 1H, NH), 5.67 (bs, 1H, NH), 5.56 (bs, 1H, NH), 4.75–4.68 (m, 1H, CαH), 4.60–4.53 (m, 1H, CαH), 4.44–4.35 (m, 3H, $CH_2O$, CαH), 4.28–4.18 (m, 1H, CαHFmoc), 3.78 (s, 3H, OMe), 3.64–3.63 (m, 1H, $CH_2N$), 3.22–3.08 (m, 1H, $CH_2N$), 2.78–2.53 (m, 4H, 2 × C≡C$CH_2$), 1.84–1.55 (m, 4H, 2 × $CH_2$), 1.76 (s, 6H, 2 × C≡CMe), 1.45 (s, 9H, Boc); $^{13}$C–NMR (75 MHz, $CD_3OD$) δ

171.8, 170.7, 170.0, 155.7, 154.8, 143.7, 143.6, 140.5, 127.5, 127.0, 125.2, 120.0, 78.3, 77.4, 75.7, 75.5, 74.7, 65.8, 55.1, 54.1, 53.7, 53.0, 52.2, 51.6, 46.8, 29.6, 28.4 (Boc), 25.9, 22.8, 21.8, 21.6, 3.51; IR ν 3297, 2972, 1655, 1529, 1250, 1166, 1053 $cm^{-1}$; HRMS (SIMS) m/z calcd for $C_{38}H_{46}N_4O_8$ $(M + Na)^+$ 687.3394, found 687.33885.

**Methyl (5*S*, 8*S*, 13*S*)13-[(*tert*-butoxycarbonyl)amino]-5-[(9*H*-9-fluorenylmethoxy)carbonyl]amino-6,14-dioxo-1,7-diaza-10-cyclotetradecyne-8-carboxylate (62).** Using the general procedure for ring closing alkyne metathesis compound **61** (30.0 mg, 43 μmol) was cyclized. After chromatography (2% $CH_2Cl_2$ in MeOH) cyclized product **62** was obtained as a white crystalline solid (9.7 mg, 15 μmol, 38%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.0 Hz, 2H, 2 × ArH), 7.59 (d, *J* = 5.3 Hz, 2H, 2 × ArH), 7.39 (t, *J* = 7.3 Hz, 2H, 2 × ArH), 7.29 (t, *J* = 7.3 Hz, 2H, 2 × ArH), 7.06 (bs, 1H, NH), 6.86 (bd, *J* = 6.8 Hz, 1H, NH), 6.03 (bd, *J* = 9.0 Hz, 1H, NHFmoc), 5.59 (bs, 1H, NHBoc), 4.74−4.65 (m, 1H, CαH), 4.53−4.47 (m, 1H, CαH), 4.42−4.34 (m, 3H, $CH_2O$, CαH), 4.26−4.20 (m, 1H, CαHFmoc), 3.82 (s, 3H, OMe), 3.04−2.85 (m, 2H, C*H*$_2$NH), 2.77−2.31 (m, 4H, 2 × C≡C$CH_2$), 2.05−1.61 (m, 4H, 2 × $CH_2$), 1.49 (m, 9H, Boc); $^{13}$C−NMR (75 MHz, $CD_3OD$) δ 171.8, 170.3, 169.9, 155.5, 154.4, 143.7, 143.6, 140.5, 127.5, 126.9, 125.2, 120.0, 79.5, 79.0, 78.6, 65.8, 53.6, 53.1, 52.2, 51.4, 46.8, 37.2, 30.3, 28.4, 25.6, 23.0, 20.2; IR ν 3338.7, 1713.1, 1650.6, 1025.0, 1003.8 $cm^{-1}$.




*General procedure for the loading of 2-chlorotrityl chloride.*

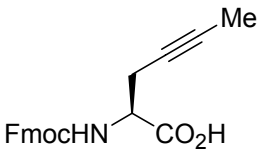



Under inert conditions, a batch of resin was allowed to swell in dry $CH_2Cl_2$. After removal of the $CH_2Cl_2$, fresh dry $CH_2Cl_2$ was added, followed by the Fmoc-protected amino acid (1.3 equiv) and DiPEA (2 equiv). After 40 min, DiPEA (2 equiv) and MeOH (5 mL) were added and the reaction was continued for 10 min. The resulting mixture was filtrated, washed using $CH_2Cl_2$ and dried. The loading was determined by measuring weight or Fmoc-titration.

*General procedure for amino acid coupling using Fmoc-NH-Resin.*
The Fmoc-protected amino acid loaded resin was treated with 20% piperidine and 0.1 M HOBt in DMF (15 mL) for 5 min and subsequently the solvent was removed (by filtration). This procedure was repeated three times. The resin was washed using DMF (3 × 1 min). Having removed the Fmoc, the resin was treated with DIC (3.3 equiv, 1 M in DMF), HOBt (3.3 equiv, 1 M in DMF), the Fmoc-AA-OH (3 equiv) and DMF and 'stirred' for approximately 40 min . The coupling was qualitatively monitored using the Kaiser test. When completed, the solvent was again removed by filtration and washed with DMF (3 × 1 min).

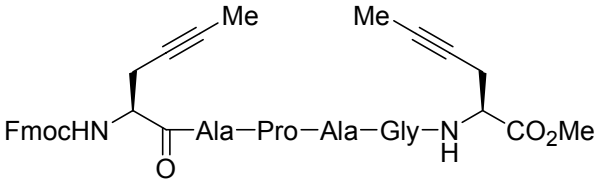
*General procedure for the cleavage from the chloritrityl-resin.*
The resin was washed twice using MeOH, twice with $CH_2Cl_2$ and twice with $Et_2O$ in order to remove all traces of DMF. Then 1% of ethanedithiol, 1% of TFA and 1% of $^iPr_3SiH$ in $CH_2Cl_2$ was added and the resin was mixed for 2 h. After filtration, the filtrate was concentrated, dissolved in MeOH and treated with $Et_2O$, causing the peptide-chain to precipitate the solution. The solid peptide was dissolved in dioxane and freeze-dried under vacuum, yielding a white solid. The corresponding methyl esters were obtained either by treatment with $CH_2N_2$ in $Et_2O$ or with in MeOH and 1.5 equiv $SOCl_2$ for 48 h.[35]

**(*S*)-2-(Fluoren-9-ylmethoxycarbonylamino)-hex-4-ynoic acid (63).**




(*S*)-2-Amino-hex-4-ynoic acid (**30**, 5.21 g, 40.9 mmol) was dissolved in water (35 mL) and $Et_3N$ (5.75 mL, 40.9 mmol) was added in one shot. Then FmocOSu (13.11 g, 38.9 mmol) was added in a solution of acetonitrile (35 mL, a clear solution was obtained by gentle heating). The pH was continuously kept below 9 by careful addition of $Et_3N$. When the pH stabilized around 9, the reaction was stirred for 10 min. The pH was lowered to 6 by addition of 2 M HCl and the volatiles was removed by reduced pressure. The mixture was acidified to pH = 2 and extracted using EtOAc (3 × 50). The organic layer was washed (brine), dried ($Na_2SO_4$) and evaporated. Further purification was performed by recrystallizing from hot EtOAc and heptane. Product **63** was obtained as white solid (13.66 g, 39.0 mmol, 96%). $[\alpha]_D$ = +62.0 (*c* = 1.15, $CH_2Cl_2$); $^1$H−NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.3 Hz, 2H, ArH), 7.65−7.59 (m, 2H, ArH), 7.41 (t, *J* = 7.5 Hz, 2H, ArH), 7.32 (t, *J* = 7.2 Hz, 2H, ArH), 5.57 (bd, *J* = 7.6 Hz, 1H, NH), 4.57−4.48 (m, 1H, CαH), 4.44−4.40 (m, 2H, $CH_2O$), 4.29−4.22 (m, 1H, CαH-Fmoc), 2.81−2.73 (m, 2H, C≡C$CH_2$), 1.80 (s, 3H, C≡CMe); $^{13}$C−NMR (75.5 MHz, $CDCl_3$) δ 174.5, 155.5,

143.4, 140.9, 127.4, 126.8, 124.9, 119.7, 79.7, 72.5, 67.3, 52.5, 47.1, 22.8, 3.7; IR ν 2971, 1716, 1517, 1449, 1220, 1056 $cm^{-1}$.




**Fmoc-Mpg-Ala-Pro-Ala-Gly-Mpg-OMe (65).** Using standard solid phase chemistry (700 mg resin, 0.496 mmol) and methyl esterfication, oligopeptide **65** (230 mg, 0.29 mmol, 59%) was obtained as a white fluffy solid. $^{1}$H−NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H, ArH), 7.65−7.56 (m, 3H, NH, 2 × ArH), 7.42−7.37 (m, 3H, NH, 2 × ArH), 7.33−7.28 (m, 3H, NH, 2 × ArH), 7.09 (bd, *J* = 7.0 Hz, 1H, NH), 5.83 (bd, *J* = 7.6 Hz, 1H, NHFmoc), 4.81−4.77 (m, 1H, CαH), 4.67−4.60 (m, 1H, CαH), 4.55−4.31 (m, 5H, 3 × CαH, $CH_2O$), 4.23 (t, *J* = 6.5 Hz, CαH-Fmoc), 4.05 (d, *J* = 15.9 Hz, 1H, $CH_2$-Gly), 3.91 (d, *J* = 15.4 Hz, 1H, $CH_2$-Gly), 3.72 (s, 3H, OMe), 3.66−3.55 (m, 2H, $CH_2N$), 2.69−2.49 (m, 4H, 2 × C≡C$CH_2$), 2.28−1.91 (m, 4H, 2 × $CH_2$), 1.75 (s, 3H, C≡CMe), 1.72 (s, 3H, C≡CMe),1.42−1.32 (m, 6H, 2 × Me-Ala); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 170.8, 170.7, 170.4, 170.3, 169.1, 168.2, 155.4, 143.2, 140.7, 127.2, 126.5, 124.5, 119.4, 79.0, 76.9, 73.3, 72.5, 67.0, 60.2, 53.2, 52.4, 51.0, 49.0, 47.3, 47.2, 46.8, 42.7, 28.7, 28.2, 25.1, 23.3, 22.3, 17.8, 17.6, 3.5, 3.4; HRMS (MALDITOF): calcd for $C_{41}H_{48}N_6O_9$ 768.3483, found 790.8989 (Ma + Na)$^+$.

**Fmoc-Mpg-Gly-Glu(O$^t$Bu)-Gly-Ala-Mpg-OH (64).** Using standard solid phase chemistry (700 mg resin, 0.496 mmol) and methyl esterfication, oligopeptide **64** (260 mg, 0.31 mmol, 62%) was obtained as a white fluffy solid. HRMS (MALDITOF): calcd for $C_{43}H_{52}N_6O_{11}$ 828.3694, found 850.8803 (M + Na)$^+$.

**Fmoc-Mpg-Gly-Ala-Pro-Ala-Gly-Ala-Mpg-OH (66).** Using standard solid phase chemistry (750 mg resin, 0.51 mmol) and methyl esterfication, oligopeptide **66** (340 mg, 0.39 mmol, 76%) was obtained as a white fluffy solid. $^{13}$C−NMR (75 MHz, DMSO-*D6*) δ 172.3, 172.0, 171.1, 170.6, 170.4, 170.3, 170.1, 167.9, 155.7, 143.6, 140.5, 131.6, 131.5, 128.5, 127.5, 127.0, 125.2, 120.0, 78.3, 77.6, 75.8, 74.7, 74.5, 67.5, 66.5, 65.9, 60.8, 59.5, 54.3, 52.4, 52.2, 49.6, 48.6, 47.9, 46.8, 46.3, 34.4, 30.0, 29.2, 28.6, 24.7, 23.5, 22.7, 22.4, 21.5, 18.6, 18.0, 17.3, 14.3, 14.2, 11.1, 3.6, 3.5; HRMS (MALDITOF): calcd for $C_{45}H_{54}N_8O_{11}$ 882.3912, found 906.7462 (M + Na)$^+$.

**Fmoc-Mpg-Ala-Gly-Ala-Pro-Ala-Gly-Ala-Gly-Mpg-OH (67).** Following the general procedure for solid phase chemistry (750 mg resin, 0.510 mmol), oligopeptide **67** (358 mg, 0.35 mmol, 70%) was obtained as a white fluffy solid.$^{13}$C−NMR (75 MHz, DMSO-*D*$_6$) δ 172.4, 172.2, 172.0, 171.6, 170.7, 170.3, 170.0, 168.6, 168.4, 167.9, 155.7, 143.6, 140.5 (2 ×), 128.2, 127.6, 127.0 (2 ×), 125.2, 125.1, 120.0, 78.4, 77.7, 75.7, 74.5, 72.3, 65.9, 60.4, 59.5, 54.1, 52.3, 51.6, 48.7, 46.9, 46.4, 43.8, 29.2, 24.7, 22.3, 21.7, 18.4, 18.2, 17.9, 3.5 (2 ×); HRMS (MALDITOF): calcd for $C_{50}H_{62}N_{10}O_{13}$ 1010.4498, found 1032.9927 $(M + Na)^+$.

**Fmoc-Mpg-Ala-Gly-Ala-Gly-Ala-Pro-Ala-Gly-Ala-Gly-Ala-Gly-Mpg-OMe (68).** Using standard solid phase chemistry (825 mg resin, 0.561 mmol) and methyl esterfication, oligopeptide **68** (600 mg, 0.47 mmol, 84%) was obtained as a white fluffy solid.[1] $^3$C−NMR (75 MHz, DMSO-*D*$_6$) δ 171.8 (3 ×), 170.9, 170.5, 170.4, 170.0, 169.7 (2 ×), 168.3, 168.1, 167.9, 167.6, 155.4, 143.4, 140.3, 127.2, 126.7, 124.9, 119.8, 78.1, 77.3, 75.5, 74.2, 65.6, 59.3, 59.2, 53.8, 52.0, 51.3, 48.3 (2 ×), 48.2, 48.1, 46.6, 46.5, 46.1, 41.9, 41.6, 41.5, 28.9, 24.4, 22.1, 21.4, 18.1 (2 ×), 17.8, 17.2, 17.1, 3.3 (2 ×); HRMS (MALDITOF): calcd for $C_{60}H_{78}N_{14}O_{17}$ 1266.5669, found 1288.988 $(M + Na)^+$.

## *5.11 References and notes*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37 KPB stands for 'kookpuntsbenzeen' (Dutch), comparable with petroleum ether 60/80 or hexanes.
38 All spectroscopic data were in accordance with previously reported literature values.
39 It was essential to maintain the temperature as low as possible due to decomposition, hence a low pressure was required.

# 6 TOWARD A SYNTHESIS OF FE399

## 6.1 Introduction

In Chapters 4 and 5, our aims were directed to the replacement of sulfur-bridges by carbon analogues in order to reach stable, conformationally rigid peptide structures. It was shown that especially ring-closing alkyne metathesis in a number of cases appeared an effective tool to introduce conformational constraints. However, biological activity foremost depends on the properties of the ensemble, not just on the isolated units. Comparison of cystine and its carbon counterpart in terms of metabolical and chemical stability focuses solely on the properties of the diamino acid unit itself.

Examples of biologically active cystine bridged peptides and comparison to the corresponding carbon analogues are rare and to a large extent summarized in Chapter 1. A relevant example is oxytocin (**1**),[1] the properties of which have recently been compared to its carbon analogue **2** (Figure 1).[2] In this example the biological activity of native oxytocin (**1**, $EC_{50}$ = 2.7 ng/mL) is diminished to 38 and 242 ng/mL for the *Z*- and *E*-analogues of **2**, respectively. Hydrogenation of the mixture to give the saturated linkage further reduced the $EC_{50}$ to 338 ng/mL.

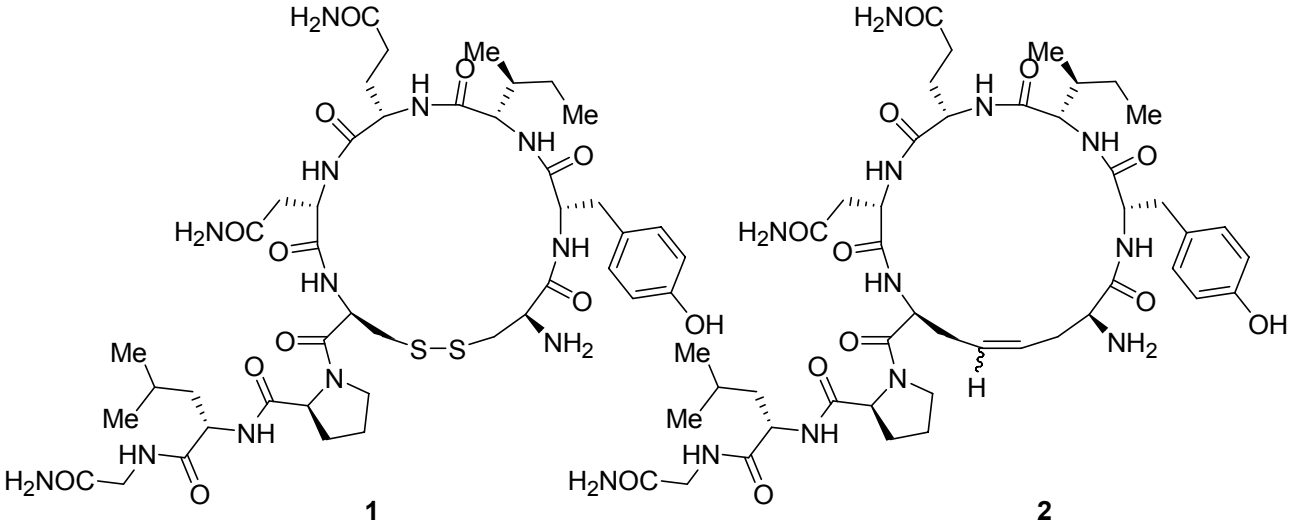
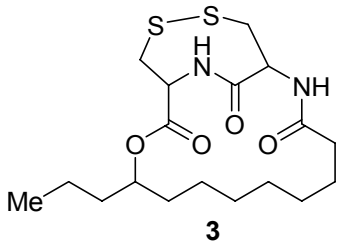



**Figure 1**. *Oxytocin (1) and its carbon analogue **2**.*

In order to further evaluate the viability of replacement of cystine bridges by their carbon counterparts in terms of biological activity, we also devised a

synthetic strategy for a cystine-containing natural product and its carbon counterpart, namely FE399 (**3**, Figure 2).[3]




**3**

**Figure 2**. *FE 399 (3).*

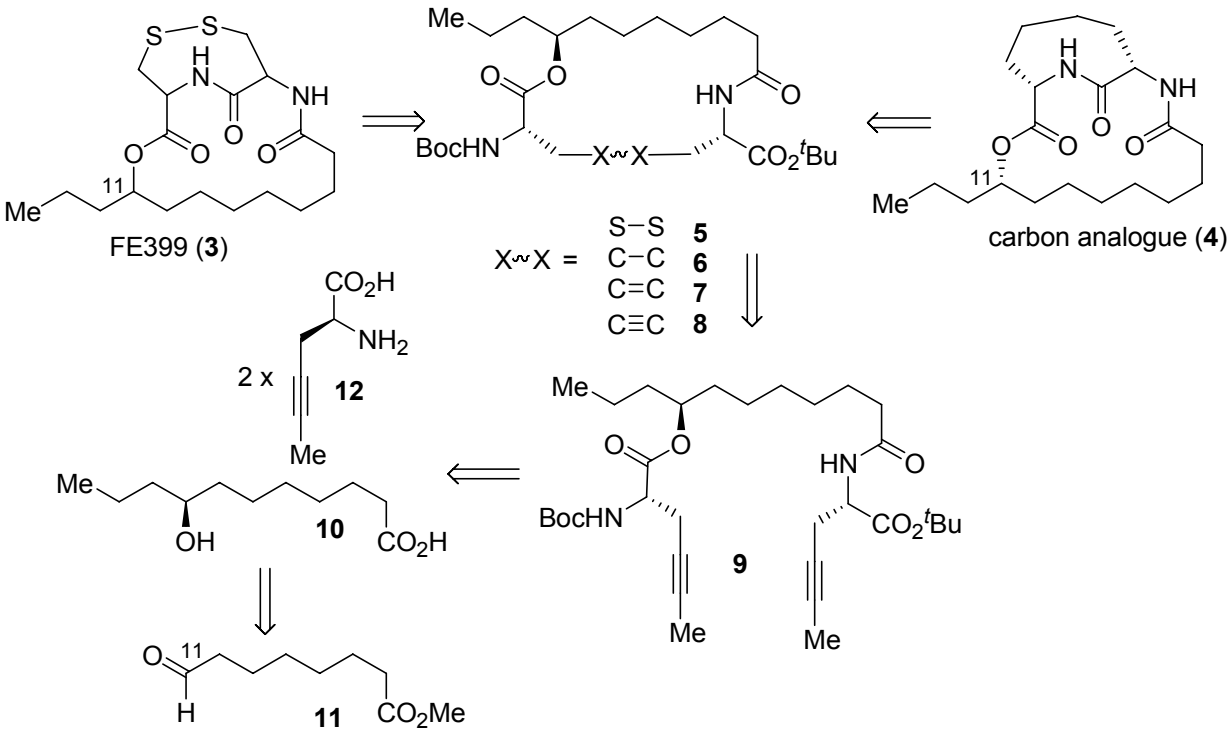
Relatively little is known of FE399 (**3**). It was isolated from cultured *Ascopchyta* sp. and indicates strong cytotoxicity and strong inhibition of tumor growth *in vivo*. Furthermore, FE399 has two conformations due to steric hindrance of the disulfide ring but the relative and absolute stereochemistry of the product is not known.

Despite the shortage of information, we considered this molecule a useful target for total synthesis and that of its carbon counterpart, especially since a short and convergent synthesis may give rise to the formation of all possible stereoisomers, and hence the opportunity to determine the absolute configuration. In this chapter a synthetic approach to both of these molecules will be discussed.

## *6.2 Retrosynthesis*

Target molecule FE399 (**3**) is a bicyclic molecule, containing two bridging cysteine residues and a functionalized 16-membered ring. Despite the fact that the stereochemical configuration at the three stereocenters is unknown, it is logic to assume that both cysteine residues possess the 'natural' (*R*)-configuration. The stereochemistry of the *n-propyl* side chain (C-11) is unknown and therefore requires a synthetic approach that allows facile inversion of this stereocenter.

The retrosynthetic approach commences with cleavage of the bridging peptide bond (*viz.* **5**-**8**). The resulting macrocyclic ring is then either formed through S-bridge formation via two cysteine residues (**5**), or via ring-closing alkyne metathesis of two acetylene containing amino acids (**8**). The advantage of alkyne metathesis would be that a triple bond is formed which can be selectively transformed into the *Z*-olefin (*viz.* **7**) via controlled Lindlar hydrogenation. Ring-closing metathesis is expected to yield a mixture of geometrical isomers, and since the presence of a *Z*-olefin is deemed necessary for the final lactam formation, the alkyne metathesis pathway is preferred.

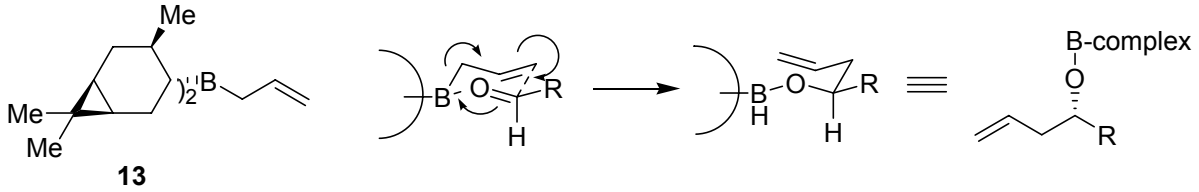



**Scheme 1.** *Retrosynthetic approach to FE399.*

Finally, synthesis of the linear cyclization precursor **9** involves coupling of the two protected amino acids **12** to the suitably functionalized enantiopure aliphatic chain **10**. In view of the unknown C-11 stereocenter, we aimed at constructing the latter fragment from aldehyde **11**, since multiple methods exist to form the desired stereocenter in both enantiomeric forms via enantioselective functionalization of the aldehyde group.

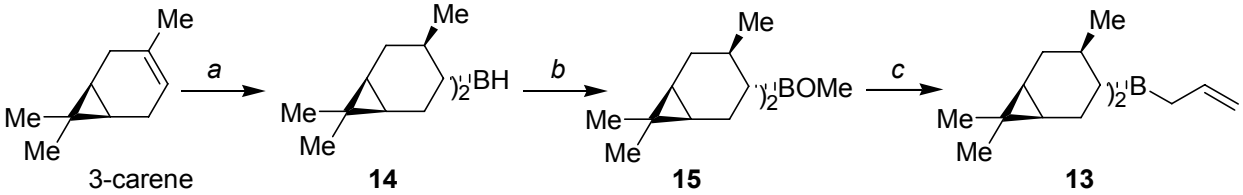
## *6.3 Synthesis of the aliphatic chain*

Asymmetric allylation of aldehydes, based on reactive borane complexes such as **13** ($^{d}Ipc_2BCH_2CH{=}CH_2$),[4] generally provides the corresponding homoallylic alcohols in excellent enantioselectivity. This readily accessible reagent reacts via a rigid chairlike transition state, in which the chiral ligand directs the outcome (Scheme 2).




**Scheme 2**. *Mechanism of the asymmetric allylation.*

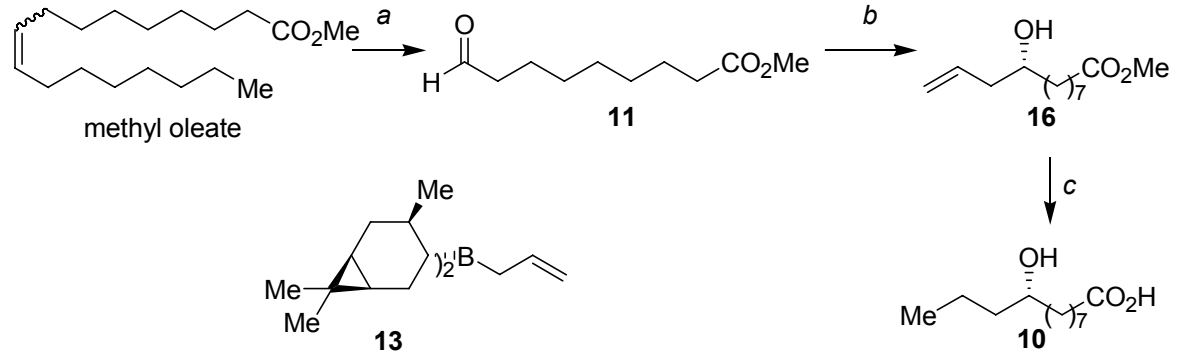
Importantly, the chirality of this reaction can be inverted by going from 3-carene to 2-carene, which can be obtained by treatment of 3-carene with base.[5] Allylboron complex **13** was obtained by treatment of 3-carene with $BH_3{\cdot}SMe_2$ (Scheme 3).[6,7] The resulting borane crystals (**14**) were collected,[8] resuspended in diethyl ether and by the addition of anhydrous methanol slowly converted into complex **15** via methanolysis.




**Scheme 3.** *Reagents and conditions: a) $BH_3{\cdot}SMe_2$; b) MeOH, $Et_2O$; c) AllylMgBr.*

In this exothermic reaction hydrogen gas was generated for approximately 2 hours. After the evolution of hydrogen gas had ceased, the solvents were removed and the resulting complex **15** was treated with allylmagnesium bromide to give the final reagent **13**.

Initial studies to form **11** were carried out starting from nonane-1,9-diol, requiring laborious protection and deprotection steps. Despite acceptable yields, a far more efficient synthesis of **11** was devised via ozonolysis of relatively cheap methyl oleate (Scheme 4). Oleate is a naturally occurring unsaturated fatty acid and its methyl ester is commercially available for 25 € $L^{-1}$. Ozonolysis, followed by reductive workup using triphenylphosphine provided **11** in 73% yield.[9]




**Scheme 4**. *Reagents and conditions: a) $O_3$, $PPh_3$, $CH_2Cl_2$ (73%); b) **13**, THF, –78 °C (52%, >99% ee.); c) i: Raney-nickel, $H_2$, ii: NaOH, MeOH, 40 °C (94%, 2 steps).*

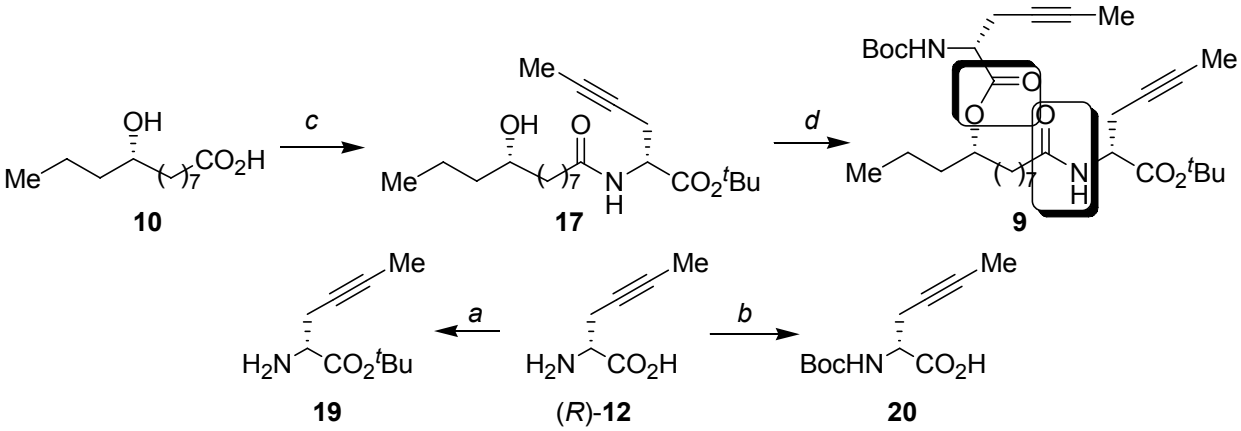
Exposure of aldehyde **11** to $^{d}Icr_2BAllyl$ (**13**) and subsequent hydrolysis of the formed borinate using $H_2O_2$ gave rise to allylic alcohol **16**. Hydrogenation catalyzed by Pd/C or $PtO_2$ surprisingly failed, while Raney-nickel provided the

desired saturated compound. This was saponified in crude form using NaOH and subsequent acid/base extraction to afford hydroxy acid **10** in pure form and high yield. The ee of compound **10** was determined to be 99% with the aid of chiral HPLC.

## *6.4 Towards an all carbon analogue of FE399*

Having established a synthesis for building block **10**, two acetylene-containing amino acids were coupled to this aliphatic chain. Initial approaches involved formation of the C-11 ester bond, followed by saponification of the methyl ester. However, the newly formed ester was as easily cleaved as the targeted methyl ester. Therefore, a somewhat different approach was pursued, in which the amide bond was formed first, provided that the ester was not base-labile. This requirement was met by using the *tert*-butyl ester-protected amino acid **19** (Scheme 5).

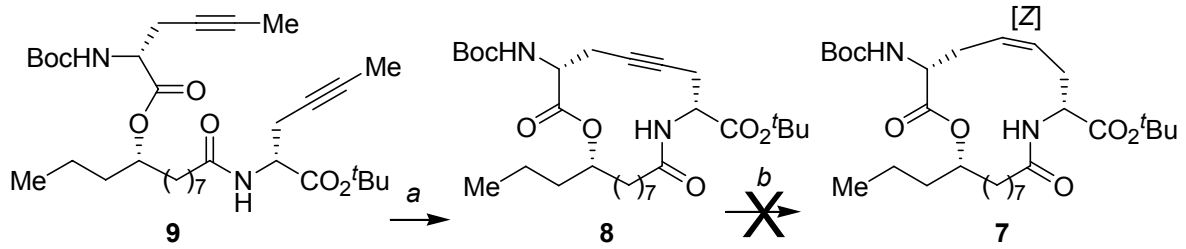
Compound **12** was esterified using condensed isobutene and a catalytic amount of sulfuric acid in a pressure flask.[10] The crude *tert*-butyl ester **19** was directly reacted with the aliphatic carboxylic acid **10** using Castro's reagent to give **17** in good yield. After purification, the second condensation with Boc-protected **20** using standard carbodiimide conditions delivered the RCAM-precursor **9** in reasonable yield. Obviously, the remaining protecting groups can be removed in a single operation using TFA. The (*R*)-configured amino acids were used since these were at that time available.




**Scheme 5**. *Reagents and conditions: a) $H_2SO_4$, isobutene (97%); b) $Boc_2O$, $NAHCO_3$, $H_2O$/1,4-dioxane (1 : 1), 100 °C, (99%); c) DiPEA, PyBOP, **19**, $CH_2Cl_2$ (79%), d) DIC, **20**, DMAP (82%).*

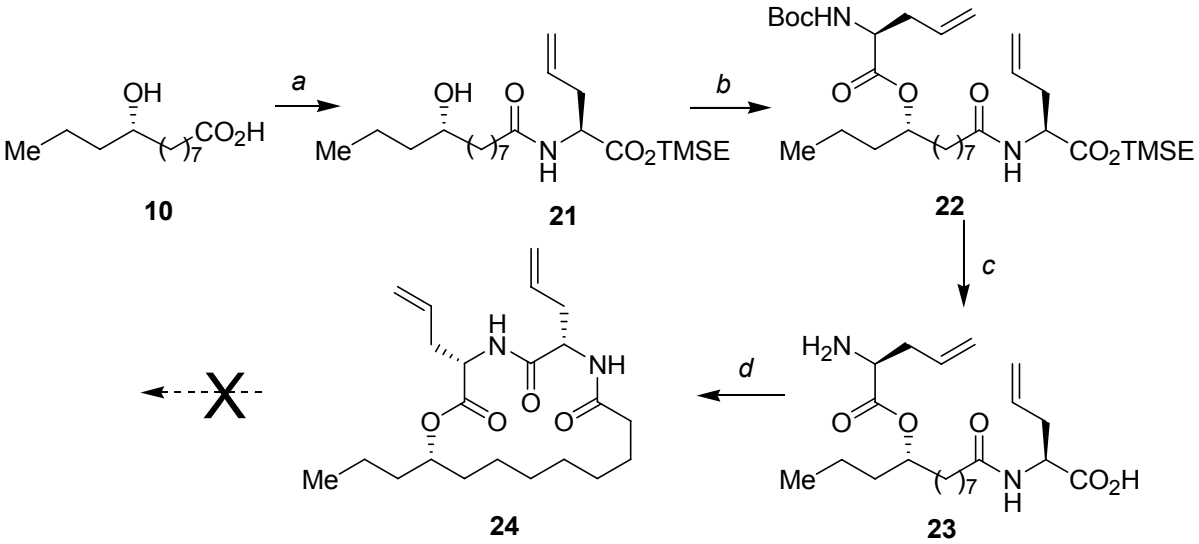
Application of RCAM using the conditions described in Section 5.3 to diacetylene **9** resulted in a successful cyclization, albeit that the yield was rather low (28%).

Furthermore, the proton signals in $^1$H−NMR were much disturbed due to the occurrence of rotamers. It was anticipated that partial hydrogenation using a Lindlar reduction would provide an easier identifiable product. Disappointingly, use of lead-poisoned palladium on calcium carbonate in the presence of quinoline resulted in unexpected and complete decomposition of starting material **8**.




**Scheme 6**. *Reagents and conditions: a) ($^t$BuO)$_3$W≡C$^t$Bu, PhMe, 80 °C (28%); b) Lindlar reduction.*

This reaction eventually was not repeated due to the inherent complexity and sensitivity of the RCAM process. Alternatively, we decided to pursue an olefin metathesis approach, since metathesis of olefins is a more straightforward reaction. Using a similar sequence as outlined in Scheme 5, 2-(*R*)-amino-4-pentenoic acid was esterified with trimethylsilylethanol, reacted with **10** using BOP-reagent and subsequently coupled to 2-(*R*)-(*tert*-butoxycarbonyl)-amino-4-pentenoic acid to give dipeptide **22** (Scheme 7).

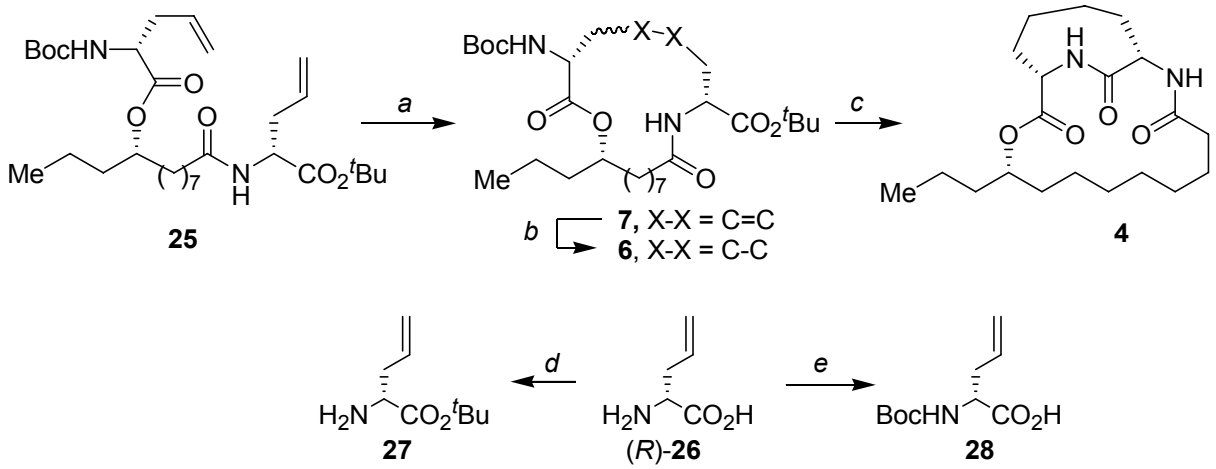



**Scheme 7**. *Reagents and conditions: a) PyBOP, 2-(trimethylsilyl)ethanol 2-(S)-4-pentenoic acid ester, $CH_2Cl_2$ (48%); b) DIC, DMAP, (S)-2-(tert-butoxycarbonylamino)-4-pentenoic acid ((*S*)-**28**), $CH_2Cl_2$ (97%); c) i: TBAF, ii: EtOAc, HCl (74%, 2 steps); d) BOP, DiPEA, $CH_2Cl_2$ (11%).*

The TMSE-protection should enable intermediate ester cleavage using TBAF. This allowed a subsequent purification after which the amine was liberated using

HCl/EtOAc and a macrolactamization was performed on amino acid **23**. Unfortunately, this latter reaction suffered from an 11% yield of **24** and even worse, RCM on **24**, carried out under a variety of conditions, did not result in any of the desired bicyclic product.

In an alternative sequence, it was decided to close the macrocyclic ring first via RCM and continue with an intramolecular peptide coupling. Using an identical procedure as for **22**, the appropriate precursor **25** was synthesized in 22% overall yield starting from the corresponding olefinic amino acid derivatives **27** and **28** (Scheme 8). Gratifyingly, ring-closing metathesis yielded the desired compound **7** as an unidentifiable mixture of *E*/*Z*-isomers and rotamers. Hydrogenation of the crude ring-closed product eventually resulted in complete conversion into compound **6**, where $^1$H–NMR-spectroscopy served to confirm the absence of the olefin.

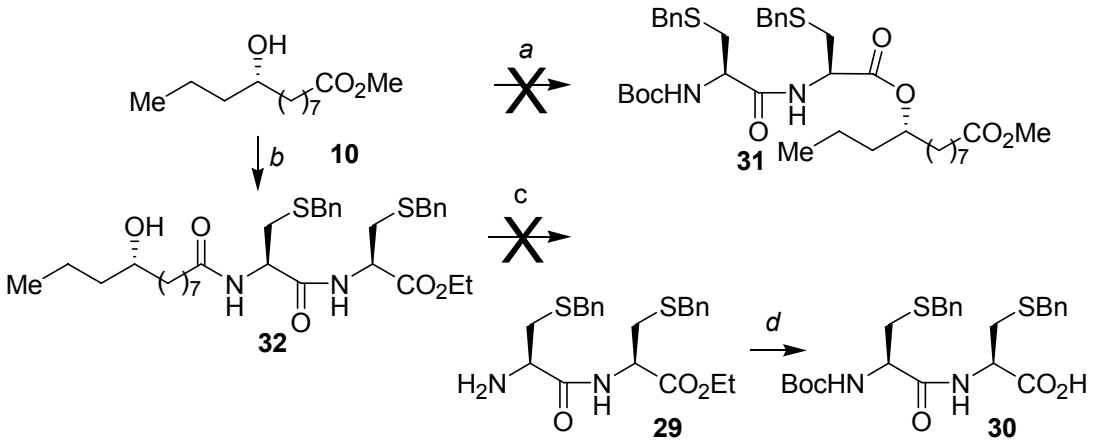



**Scheme 8**. *Reagents and conditions: a) 2nd gen Grubbs cat, PhMe, 80 °C (59%); b) H₂, Pd/C, MeOH, c) i: TFA, ii: fluorocyanuric acid (13%, 3 steps); d) H₂SO₄, isobutene (97%); e) Boc₂O, NaHCO₃, H₂O/1,4-dioxane (99%).*

Continuing the sequence by TFA-mediated deprotection of the amine and ester function and subsequent lactamization using *in situ* acyl fluoride formation with fluorocyanuric acid led to product **4**, the formation of which was confirmed using determination of the exact mass.[11] The amount of material, however, was insufficient for extensive characterization and comparison of its properties to those of FE399.

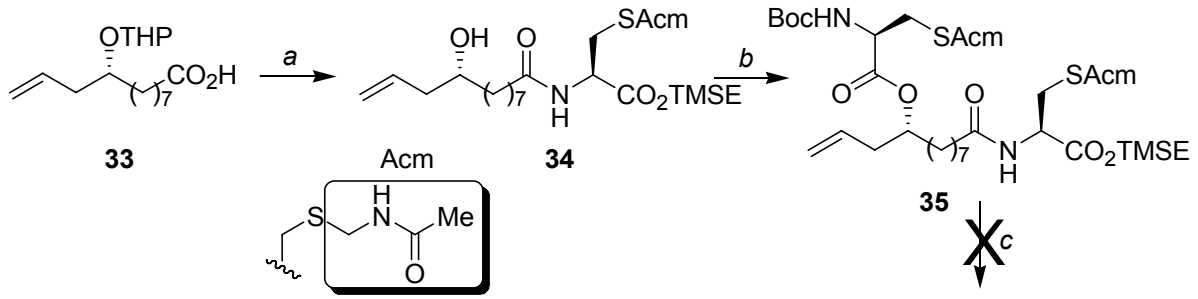
## 6.5 Towards FE399

The synthetic strategy to FE399 posed additional difficulties related to the required orthogonal protecting groups for the sulfur atoms. Initial approaches relied on combining the commercially available dipeptide **29** with the previously described aliphatic chain **10**. Markedly, as shown in Scheme 9, the amide bond formation-coupling using the typical DIC conditions was unsuccessful. The more successful amide installment which led to **32** might eventually lead to the required lactone. However, prior to this lactonization the cystine-formation was investigated since the weak ester bond might be incompatible with cystine-formation. However, upon subjection of **32** to typical Birch conditions (lithium in ammonia) no recoverable products were found. It must also be noted that the hydrolysis and subsequent lactonization of **32** is known to give severe racemization at the cysteine moieties.



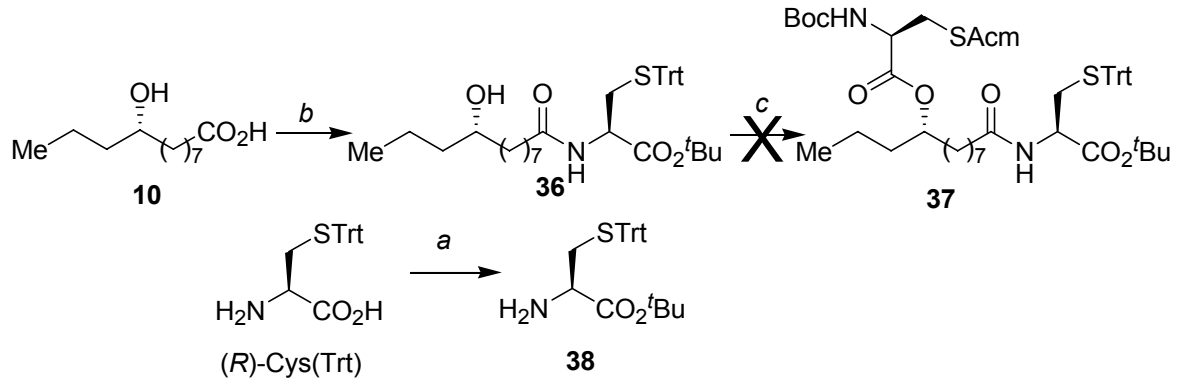

**Scheme 9.** *Reagents and conditions: a)* ***30****, DIC, DMAP, $CH_2Cl_2$; b) i: NaOH, MeOH; ii: PyBOP, DiPEA,* ***29****, $CH_2Cl_2$ (38%); c) Li, $NH_{3(l)}$; d) i: $Boc_2O$, $Et_3N$; ii) 2 M LiOH (82%, 2 steps).*

As an alternative, the previously discussed TMSE-ester strategy was followed, which is illustrated in Scheme 10.[12]




**Scheme 10.** *Reagents and conditions: a) i: PyBOP, DiPEA, Cys(Acm)-OTMSE, $CH_2Cl_2$; ii) pTSA, MeOH (30%, 2 steps); b) DIC, DMAP, Boc-Cys(Acm)-OH, $CH_2Cl_2$ (29%); c) TBAF.*

The main advantage of the acetamidomethyl (Acm) group is its stability under the most common deprotection conditions. It can be easily removed by treatment with mercury(II)-salts, and it allows iodine-mediated oxidation without deprotection. Unfortunately, after TBAF-assisted deprotection the desired product was inseparable from byproducts that were formed in this reaction.
Attempts to switch to different ester protective groups were not very successful. As already mentioned, the methyl ester cannot be hydrolyzed in the presence of the secondary ester. A more labile 2-Ts ethyl ester based cysteine could not be used because of its incompatibility with the lactam forming step. Furthermore, in the presence of the sulfur groups use of a benzyl ester is not possible. However, when free *S*-trityl-protected cysteine (Cys(Trt)) was reacted with DCC/$^t$BuOH indeed a *tert*-butyl ester was isolated and subsequently attached to acid **10** in a reasonable yield of 58%.




**Scheme 11**. *Reagents and conditions: a) CuCl, $^t$BuOH, DCC; b) PyBOP, DiPEA, $CH_2Cl_2$ (58%, 2 steps); c) Boc-Cys(Acm)-OH, DIC, $Et_3N$, $CH_2Cl_2$.*

At this stage, the ester bond formation between Boc-Cys(Trt)-OH or Boc-Cys(Acm)-OH and the secondary alcohol of **36** was ineffective for unknown reasons. Since in early stages we experienced much difficulties associated with this particular alcohol,[13] the trityl group could be responsible for the diminished activity. The aliphatic chain could fold itself in such a fashion that the secondary alcohol of **36** is shielded by the rest of the molecule.

## *6.6 Conclusions*

The synthesis of FE399 gave more difficulties than we initially anticipated. The originally planned retrosynthetic approach based on metathesis failed to give the desired carbon mimic in the case of alkyne substituents, but was eventually successful in the case of alkene metathesis. This led to a saturated carbon analogue of FE399, which due to the small amounts of isolated product was

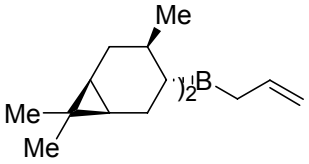
insufficient for evaluation of its properties. Furthermore, in pursuing the natural product itself, unfortunately a suitable sequence was not found as a result of complications caused by the protecting group manipulations.

## 6.7 Acknowledgements

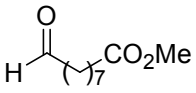
Bas Gruijters and Leon Stelten are kindly acknowledged for their contributions to this chapter.

## 6.8 Experimental section

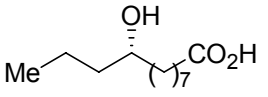
For general experimental details, see: Section 2.8.




**$^{d}$Icr$_2$BAllyl (13)**.[14] 3-Carene (42 g, 308 mmol) was added drop wise to a cooled (0 °C) solution of $BH_3$·DMS (10 mL, 106 mmol) in $CH_2Cl_2$ (50 mL). The clear solution was placed in the fridge for 48 h after which the suspension was filtered under an argon atmosphere and washed with cold $Et_2O$. The isolated white solid (19.5 g, 68 mmol) was directly transferred to a flask, resuspended in $Et_2O$ (100 mL) and MeOH (10 mL) was added. The suspension was stirred until the solution became clear, after which the solvent was removed using vacuum evaporation. THF (150 mL) was added and a 2 M solution of allylmagnesium bromide (34.0 mL, 68.0 mmol) was added dropwise at –78 °C. The temperature was slowly raised to RT after which the resulting solution was used for follow-up chemistry.




**Methyl 9-oxononanoate (11)**.[14] A solution of methyl oleate (20.1 mL, 59 mmol) in $CH_2Cl_2$ (200 mL) was cooled to −78 °C. Ozone was bubbled through the solution until a faint blue color remained. After removal of the excess ozone using nitrogen, $PPh_3$ (17.4, 66.4 mol) was added. The solvent was stripped and $Et_2O$ (50 mL) was added. After filtration the solution was distilled (92 °C, 90 mbar) resulting in **11** as a clear oil (8.3 g, 44.6 mmol, 83%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H, CH=O), 3.67 (s, 3H, OMe), 2.47−2.43 (m, 2H, $CH_2$CH=O), 2.30 (t, $J$ = 7.4 Hz, 2H, $CH_2CO_2$Me), 1.62−0.88 (m, 10H, $CH_2$).



**9-(*S*)-Hydroxydodecanoic acid (10)**. To a stirred solution of $^{d}$Icr$_2$BAllyl (3.90 g, 11.6 mmol) in THF (150 mL) at –78 °C was added aldehyde **11** (2.00 gram, 10.9 mmol) dropwise over a period of 20 min. The mixture was stirred at –78 °C after which it was slowly warmed to room temperature. The excess of borane-complex was oxidized by addition of $H_2O_2$ (35%, 100 mL) and NaOH (10M, 2 mL) and subsequent refluxing for 2 h. Excess of $H_2O_2$ was destroyed by careful addition of aqueous saturated $Na_2S_2O_3$ and the mixture was extracted using toluene (3 × 100 mL). The organic layer was washed (brine), dried ($MgSO_4$) and evaporated. Further purification using flash chromatography (15% EtOAc in heptane) yielded desired compound **10** as a colorless oil (1.30 g, 5.5 mmol, 52%). Ee: >99%;[15] $^1$H–NMR (400 MHz, $CDCl_3$) δ 5.85–5.68 (m, 1H, =CH), 5.10–5.00 (m, 2H, =$CH_2$), 3.62–3.51 (m, 1H, C*H*OH), 3.59 (s, 3H, OMe), 2.30–2.16 (m, 3H, $CH_2$COO, $CH_2$), 2.13–2.00 (m, 1H, $CH_2$), 1.90–1.78 (m, 1H, $CH_2$), 1.62–1.47 (m, 2H, $CH_2$), 1.44–1.20 (m, 10H, 5 × $CH_2$); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 174.2, 134.8, 117.7, 70.5, 51.3, 41.8, 36.6, 33.9, 29.3, 29.1, 28.9, 25.4, 24.8.

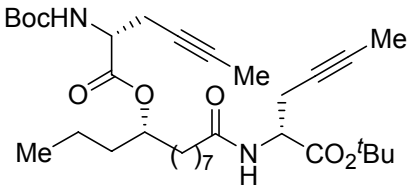
A solution of **16** (2.12 g, 9.30 mmol) in MeOH (1 M $NH_3$, 30 mL) was treated with Raney-nickel (aqueous suspension, 1 mL) upon which hydrogen was bubbled through the solution. After 2 h the suspension was filtered over Celite and the filtrate was evaporated. The resulting oil was dissolved in MeOH (20 mL), 2 M NaOH (20 mL) was added and the mixture was heated at 40 °C for 2 h. After cooling down the reaction was extracted with EtOAc. The aqueous phase was acidified (pH < 2) and extracted using $Et_2O$. The ether layer was concentrated, yielding **10** as a clean oil (1.89 g, 8.78 mmol, 94%). $^1$H–NMR (400 MHz, $CDCl_3$) δ 7.15 (bs, 1H, OH), 3.65–3.58 (m, 1H, C*H*OH), 2.32 (t, *J* = 7.8 Hz, 2H, C*H*$_2$$CO_2$H), 1.65–1.58 (m, 2H, $CH_2$), 1.47–1.25 (m, 14H, 7 × $CH_2$), 0.93 (t, *J* = 6.8 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 178.2, 71.4, 39.1, 36.9, 33.8, 29.2, 29.0, 28.8, 25.3, 24.5, 18.6, 14.0; IR ν 3400, 2928, 2855, 1708, 1223, 612 cm$^{-1}$; HMRS (FAB)$^+$ calcd for $C_{12}H_{25}O_3$ 217.18037, found 217.1804.

**2-*R*-(9-*S*-Hydroxy-dodecanoylamino)-hex-4-ynoic acid *tert*-butyl ester (17)**. A suspension of (*R*)-2-amino-hex-4-ynoic acid ((*R*)-**12**, 250 mg, 1.97 mmol) in 1,4-dioxane (10 mL) was cooled to −78 °C in a pressurizable bottle.[16] Then $H_2SO_4$ (250 μL) was added and isobutene (~10 mL) was condensed in this flask. After sufficient isobutene was added, the bottle was sealed, allowed to warm to RT and stirred for 3 days. After cooling to −78 °C, the

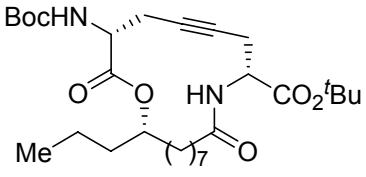
bottle was opened and allowed to warm to ambient temperature. The mixture was added to aqueous NaOH (0.5 M, 20 mL) and subsequently extracted with $Et_2O$ (3 × 20 mL). After concentration, the crude product **19** (350 mg, 1.91 mmol, 97%) was obtained.

To a stirred solution of acid **10** (246 mg, 1.13 mmol) in $CH_2Cl_2$ (20 mL) were added BOP (400 mg 0.91 mmol) and (*R*)-2-aminohex-4-ynoic acid *tert*-butyl ester (**19**, 150 mg, 0.82 mmol) and the resulting mixture was stirred overnight. The solvent was evaporated and further purified using flash chromatography (35% EtOAc in heptane) delivered peptide **17** as a clear oil (279 mg, 0.71 mmol, 89%). $[\alpha]_D$ = –7.6 (*c* = 0.41, $CH_2Cl_2$) $^1$H–NMR (400 MHz, $CDCl_3$) δ 6.22 (bd, *J* = 8.0 Hz, 1H, NH), 4.58–4.54 (m, 1H, CαH), 3.57 (m, 1H, C*H*OH), 2.70–2.55 (m, 2H, C≡C$CH_2$), 2.22 (t, *J* = 7.2 Hz, 2H, $CH_2$CO), 1.74 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.64–1.58 (m, 3H, $CH_2$), 1.46 (s, 9H, $CMe_3$), 1.46–1.37 (m, 6H, $CH_2$), 1.33–1.25 (m, 7H, $CH_2$), 0.93 (t, *J* = 6.8 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 172.2, 169.6, 82.3, 82.3, 78.6, 73.6, 71.6, 51.3, 39.8, 37.5, 36.7, 29.6, 29.4, 29.2, 28.0, 25.7, 23.2, 19.0, 14.3, 3.6; IR ν 3299, 2927, 2856, 1734, 1653, 1539, 1368, 1157 $cm^{-1}$.

**2-(*R*)-[9-*S*-(2-(*R*)-*tert*-Butoxycarbonylamino-hex-4-ynoyloxy)-dodecanoylamino]-hex-4-ynoic acid *tert*-butyl ester (9)**. To a stirred solution of alcohol **17** (154 mg, 0.40 mmol) in $CH_2Cl_2$ (10 mL) were added dropwise DIC (82 µl, 0.53 mmol), (*R*)-2-(*tert*-butoxycarbonyl)aminohex-4-ynoic acid (119 mg, 0.53 mmol) and DMAP (15 mg, 0.15 mmol) and the reaction was stirred overnight. After evaporation and further purification using flash chromatography (2% MeOH in $CH_2Cl_2$) compound **9** (196 mg, 0.33 mmol) was obtained in 82% yield. $^1$H–NMR (400 MHz, $CDCl_3$) δ 6.24 (bd, *J* = 7.6 Hz, 1H, NH), 5.35–5.30 (m, 1H, NHBoc), 4.96–4.90 (m, 1H, CαH), 4.60–4.54 (m, 1H, CαH), 4.42–4.37 (m, 1H, CαH), 2.76–2.45 (m, 4H, 2 × C≡C$CH_2$), 2.23 (t, *J* = 7.2 Hz, 2H, $CH_2$CO), 1.754 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.751 (t, *J* = 2.4 Hz, 3H, C≡CMe), 1.65–1.22 (m, 16H, 8 × $CH_2$), 1.48 (s, 9H, $CMe_3$), 1.46 (s, 9H, Boc), 0.90 (t, *J* = 7.6 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 172.9, 170.5, 169.6, 154.8, 82.2, 79.8, 78.9, 78.6, 75.7, 73.3, 52.5, 51.3, 36.7, 36.3, 34.2, 29.4, 29.3, 29.2, 28.4, 28.0, 25.7, 25.3, 23.4, 23.2, 18.5, 14.2, 3.7, 3.6.

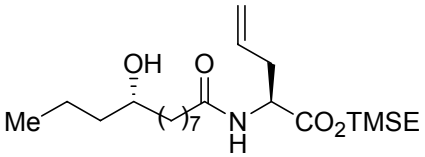


***tert*-Butyl 3-(*R*)-[(*tert*-butoxycarbonyl)amino]-2,10-dioxo-20-(*S*) propyl-1-oxa-9-aza-5-cycloicosyne-8-(*R*)-carboxylate (8).**

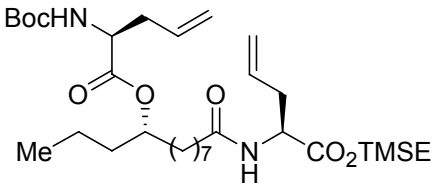



To peptide **9** (81.0 mg, 0.137 mmol) and ($^t$BuO)$_3$W≡C$^t$Bu (8.0 mg, 0.016 mmol) was added freshly distilled toluene (20 mL) and the mixture was heated at 80 °C for 3 h. After evaporation and further purification (2% MeOH in $CH_2Cl_2$) product **8** was isolated as a white foam (21.2 mg, 0.039 mmol, 28%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.19−7.16 (m, 1H, NH), 6.26−6.22 (m, 1H, NHBoc), 5.43−4.93 (m, 1H, CαH), 4.91−4.78 (m, 1H, CαH), 4.65−4.50 (m, 1H, C*H*O), 2.73−2.43 (m, 4H, 2 × C≡C$CH_2$), 2.06−2.05 (m, 2H, C$H_2$CO), 1.79−1.22 (m, 16H, aliphatic chain), 1.50 (s, 9H, Boc), 1.47 (s, 9H, $CMe_3$), 0.90 (t, *J* = 7.3 Hz, 3H, Me).

**2-(*S*)-[9-*S*-Hydroxydodecanoylamino]-4-pentenoic acid 2-(trimethylsilyl)ethyl ester (21)**. Using the coupling procedure as described for **17**, (2-(trimethylsilyl)ethyl) 2-(*S*)-4-pentenoic acid ester (73 mg, 0.34 mmol) and acid **10** (67 mg, 0.309 mmol) were coupled, resulting in a clear oil ( 120 mg, 0.25, 80%). $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.96 (d, *J* = 8.0 Hz, 1H, NH), 5.71−5.60 (m, 1H, CH=), 5.10−5.07 (m, 2H, $CH_2$=), 4.68−4.62 (m, 1H, CαH), 4.25−4.20 (m, 2H, $CH_2O$), 3.60 (s, 1H, C*H*OH), 2.60−2.48 (m, 2H, $CH_2CH$=), 2.22 (t, *J* = 7.6 Hz, 2H, $CH_2CON$), 1.60−0.93 (m, 19H, $CH_2$), 1.05−1.00 (m, 2H, $CH_2Si$), 0.07 ($SiMe_3$); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 172.0, 171.3, 131.7, 118.6, 63.6, 51.2, 36.4, 32.3, 29.4, 29.0, 17.3, −1.6.



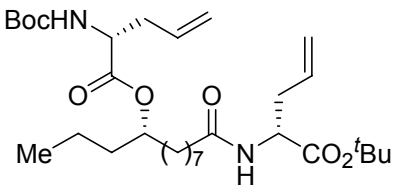

**2-(*S*)-[9-*S*-(2-(*S*)-*tert*-Butoxycarbonylamino-4-pentenoyloxy)dodecanoyl amino]-4-pentenoic acid 2-(trimethylsilyl)ethyl ester (22).**




Using the described protocol for **9**, 2-(*S*)-(*tert*-butylcarbonyl)-amino-4-pentenoic acid (244 mg, 1.14 mmol) and **21** (470 mg, 1.14 mmol) were coupled. After isolation, **22** (670 mg, 1.09 mmol, 97%) was obtained as a clear oil. $[\alpha]_D$ = +7.0 (*c* = 1.17, $CH_2Cl_2$); $^1$H−NMR (300 MHz, $CDCl_3$) δ 5.96 (d, *J* = 7.3 Hz, 1H, NH), 5.71−5.62 (m, 2H, 2 × C*H*=C), 5.13−5.07 (m, 4H, 2 × C=C$H_2$), 5.10 (d, J = 7.4 Hz, 1H, NHBoc), 4.93−4.89 (m, 1H, CαH), 4.67−4.61 (m, 1H, CαH), 4.37−4.32 (m, 1H, C*H*O), 4.25−4.21 (m, 2H, $CH_2O$), 2.60−2.44 (m, 2H, $CH_2C$=), 2.52−2.45 (m, 2H, C$H_2$C=), 2.20 (t, *J* = 7.8 Hz, 2H, C$H_2$CON), 1.70−1.22 (m, 16H, aliphatic chain), 1.47 (s, 9H, Boc), 1.04−1.00

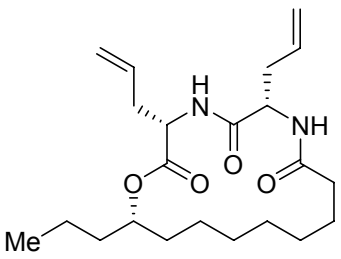
(m, 2H, $CH_2$TMS), 0.90 (t, *J* = 7.3 Hz, 3H, Me), 0.07 (s, 9H, $SiMe_3$); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 171.9, 171.3, 131.7 (2×), 118.6 (2×), 75.2, 63.6, 52.7, 51.2, 36.4, 36.3, 35.9, 33.7, 29.0, 28.9 28.1, 25.3, 18.3, 17.3, 13.8, −1.6; MS (*m*/*z*): $C_{32}H_{58}N_2O_7Si$ $(M + H)^+$ 611, 511, 483, 368, 296, 188, 136, 116, 70.

**2-(*R*)-[9-*S*-(2-(*R*)-*tert*-Butoxycarbonylamino-4-pentenoyloxy)dodecanoyl amino]pent-4-enoic acid *tert*-butyl ester (25).**



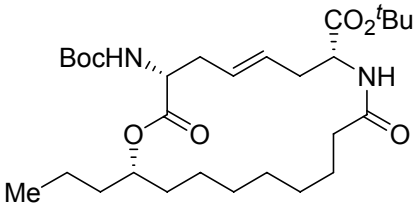

Using the method described for **17**, (*R*)-2-amino-4-pentenoic acid (400 mg, 3.48 mmol) was protected as its corresponding *tert*-butyl ester. The unpurified crude product **27** was used for further reactions. $^1$H–NMR (400 MHz, $CDCl_3$) δ 5.81–5.69 (m, 1H, CH=C), 5.19–5.14 (m, 2H, $=CH_2$), 3.47–3.41 (m, 1H, CαH), 2.52–2.42 (m, 1H, $CH_2$), 2.41–2.37 (m, 1H, $CH_2$), 1.45 (s, 9H, $CMe_3$). Upon condensation with **10** (243 mg, 1.13 mmol) and flash chromatography (33% EtOAc in heptane) the intermediate product was isolated as an oil (237 mg, 0.89 mmol, 78%). $^1$H–NMR (400 MHz, $CDCl_3$) δ 6.00 (bs, 1H, NH), 5.75–5.62 (m, 1H, C=CH), 5.14–5.11 (m, 1H, $C=CH_2$), 5.09–5.06 (m, 1H, $C=CH_2$), 4.60–4.54 (m, 1H, CαH), 3.63–3.56 (m, 1H, C*H*OH), 2.63–2.53 (m, 1H, $CH_2C=$), 2.51–2.43 (m, 1H, $CH_2C=$), 2.19 (dt, *J* = 1.9 Hz, 7.8 Hz, 2H, $CH_2CO$), 1.74–1.24 (m, 16H, aliphatic chain), 1.46 (s, 9H, $CMe_3$), 0.92 (t, *J* = 6.8 Hz, 3H, Me); $^{13}$C–NMR (50 MHz, $CDCl_3$) δ 172.6, 171.0, 132.3, 118.6, 82.0, 71.3, 51.9, 51.7, 39.4, 17.2, 36.6, 36.3, 29.3, 29.1, 28.9, 27.8, 25.4, 18.6, 14.0; IR ν 3316, 2928, 2855, 1724, 1656, 1532, 1368, 1154 $cm^{-1}$. Using the protocol as described for **9**, the previously obtained alcohol (185 mg, 0.50 mmol) was condensed with 2-(*R*)-(*tert*-butoxycarbonyl)amino-4-pentenoic acid ((*R*)-**28**, 125 mg, 0.58 mmol). Product **25** (80 mg, 0.14 mmol, 28%) was isolated after flash chromatography (2% MeOH in $CH_2Cl_2$). $^1$H–NMR (400 MHz, $CDCl_3$) δ 6.00 (d, *J* = 7.3 Hz, 1H, NH), 5.75–5.63 (m, 2H, 2 × C*H*=C), 5.17–5.06 (m, 4H, 2 × C=C*H*$_2$), 5.10 (d, *J* = 7.4 Hz, 1H, NHBoc), 4.93–4.88 (m, 1H, CαH), 4.60–4.55 (m, 1H, CαH), 4.37–4.32 (m, 1H, C*H*O), 2.62–2.54 (m, 2H, $CH_2C=$), 2.52–2.45 (m, 2H, C*H*$_2$C=), 2.19 (t, *J* = 7.8 Hz, 2H, C*H*$_2$CON), 1.70–1.22 (m, 16H, aliphatic chain), 1.47 (s, 9H, Boc), 1.44 (s, 9H, $CMe_3$), 0.90 (t, *J* = 7.3 Hz, 3H, Me); $^{13}$C–NMR (75 MHz, $CDCl_3$) δ 172.2, 171.5, 170.6, 154.9, 132.2 (2 ×), 118.8, 118.6, 82.1, 79.6, 75.4, 53.1, 51.8, 36.9, 36.7, 36.2, 34.2, 29.3, 29.2, 28.4 (3 ×), 28.2 (3 ×), 25.7, 25.3, 18.7, 14.1; HRMS $(FAB)^+$ calcd for $C_{31}H_{55}O_7N_2$ 567.40093, found 567.4011 $(M + H)^+$.

**3-(*R*),6-(*R*)-Diallyl-18-propyl-1-oxa-4,7-diazacyclooctadecane-2,5,8-trione**



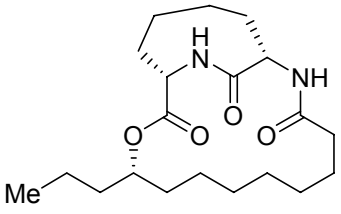
**(24).** Product **25** (155 mg, 0.303 mmol) was treated with 2M HCl in EtOAc (10 mL) for 2h upon which the volatiles were removed. The crude unprotected product was dissolved in $CH_2Cl_2$ and BOP ( 323 mg, 0.731 mmol) and DiPEA (198 mg, 1.52 mmol) were added. After stirring overnight the solvent was stripped and further purification using flash chromatography (2% MeOH in $CH_2Cl_2$) yielded product **24** as a sticky oil (13 mg, 0.033 mmol, 11%, 2 steps). $^1$H−NMR (300 MHz, $CDCl_3$) δ 6.28 (d, *J* = 7.0 Hz, 1H, NH), 6.15 (d, *J* = 6.8Hz, 1H, NH), 5.81−5.61 (m, 2H, 2 × =CH), 5.13−5.07 (m, 4H, 2 × =$CH_2$), 4.91−4.63 (m, 3H, 2 × CαH, C*H*O), 2.61−0.82 (m, 21 H); $^{13}$C−NMR (50 MHz, $CDCl_3$) δ 172.4, 169.9, 165.2, 132.3, 131.7, 119.0, 118.4, 75.6, 52.2, 51.7, 37.0, 36.5, 32.8, 26.7, 25.8, 24.8, 22.5, 18.6, 18.5, 13.8, 13.7.

***tert*-Butyl 3-(*R*)-[(*tert*-butoxycarbonyl)amino]-2,10-dioxo-20-(*S*) propyl-1-oxa-9-aza-5-cycloicosene-8-(*R*)-carboxylate (7).**



To a stirred solution of **25** (80 mg, 0.17 mmol) in anhydrous toluene (10 mL) was added Grubbs catalyst (2$^{nd}$ gen, 8 mg, 9 μmol). The mixture was heated at 60 °C for 8 h. After evaporation and further purification using flash chromatography (33% EtOAc in heptane) product **7** was isolated as an off-white solid (45 mg, 0.10 mmol, 59%); $^1$H−NMR (300 MHz, $CD_3OD$) δ 5.57−5.40 (m, 2H, 2 × =CH), 4.89−4.84 (m, 1H, CαH), 4.46−4.38 (m, 1H, CαH), 4.14−4.08 (m, 1H, C*H*OH), 2.55−2.39 (m, 4H, $CH_2C$=), 2.19−2.15 (m, 2H, $CH_2CO$), 1.69−1.23 (m, 16H), 1.46 (s, 9H, Boc), 1.44 (s, 9H, $CMe_3$), 0.91 (t, *J* = 7.2 Hz, 3H, Me); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 174.4, 172.9, 170.3, 158.6, 128.3, 127.7, 81.0, 74.8, 53.7, 51.3, 35.7, 34.5, 34.2, 32.5, 28.8, 27.5, 27.2, 26.7 (3 ×), 26.4 (3 ×), 24.4, 23.8, 17.8, 12.4; IR ν 2934, 1721, 1670, 1482, 1180, 1071, 1054, 1032 cm$^{-1}$.

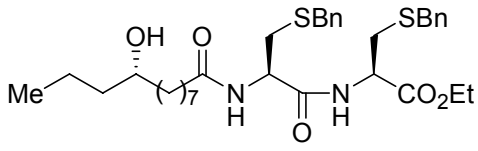
**(*R*,*R*,*R*)-11-Propyl-12-oxa-2,20-diaza-bicyclo[12.4.2]icosane-3,13,19-trione**



**(4).** Through a suspension of **7** (27 mg, 61 μmol) and Pd/C (10%, 20 mg) in MeOH (10 mL) was bubbled hydrogen gas for 2 h. After filtration over Celite product **6** was obtained as a white solid.[17] The product was dissolved in TFA (50% in $CH_2Cl_2$, 4 mL) and stirred for 4 h. After

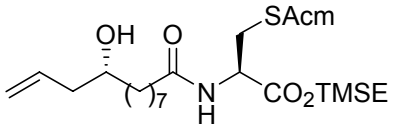
evaporation, $CH_2Cl_2$ (3 mL), fluorocyanuric acid (16 mg, 0.122 mmol) and DiPEA (110 µL, 0.06 mmol) were added and stirring was continued for 16 h. Upon evaporation silica gel chromatography was performed (60% EtOAc in heptane) resulting in **4** as a white solid (2.9 mg, 7.9 µmol, 13%). HRMS (FAB$^+$): calcd for $C_{20}H_{34}N_2O_4$ 367.25968, found 367.2597 $(M + H)^+$.

**Ethyl 3-(benzylsulfanyl)-2-(*R*)-(3-(benzylsulfanyl)-2-(*R*)-[(9-(*R*)-hydroxydodecanoyl)amino]propanoylamino)propanoate (32).**



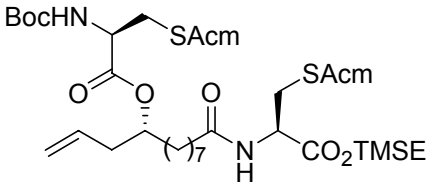

To a stirred solution of acid **10** (221 mg, 1.02 mmol) in $CH_2Cl_2$ (10 mL) were added DiPEA (440 µL, 2.44 mmol), PyBOP (650 mg, 1.20 mmol) and $H_2N$-Cys(Bzl)-Cys(Bzl)-OEt (530 mg, 1.22 mmol) respectively. After stirring for 16 h the solvent was removed and further purification using flash chromatography (2% MeOH in $CH_2Cl_2$) was performed. Product **32** was isolated as an amorphous solid (250 mg, 0.39 mmol, 38%). $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.37−7.21 (m, 10H, 2 × Ar), 7.09 (d, *J* = 7.7 Hz, 1H, NH), 6.35 (d, *J* = 7.4 Hz, 1H, NH), 4.74−4.68 (m, 1H, CαH), 4.57−4.51 (m, 1H, CαH), 4.19 (q, *J* = 7.1 Hz, 2H, $OCH_2$), 3.79 (s, 2H, $ArCH_2S$), 3.70 (s, 2H, $CH_2S$), 3.61−3.57 (m, 1H, C*H*OH), 2.91−2.69 (m, 4H, 2 × $CH_2S$), 2.18 (t, *J* = 7.6 Hz, 2H, CON), 1.65−1.25 (m, 16H, 8 × $CH_2$), 0.92 (t, *J* = 6.7 Hz, 3H, Me); $^{13}$C−NMR (75 MHz, $CD_3OD$) δ 174.5, 170.9, 170.0, 137.7, 137.5, 133.9, 129.7, 128.3, 127.6, 126.3, 125.1, 125.0, 116.8, 109.7, 71.9, 70.1, 60.9, 51.7, 38.7, 36.7, 35.3, 35.2, 32.3, 31.6, 28.9, 28.7, 28.4, 25.1, 25.0, 18.0, 12.7, 12.6; IR ν 3305, 2924, 2882, 1744, 1631, 1544, 1213, 1032 cm$^{-1}$; HRMS (EI) calcd for $C_{34}H_{50}N_2O_5S_5$ 630.31611, found 653.30588 $(M + Na)^+$.

**2-(1,1,1-Trimethylsilyl)ethyl 3-[(acetylamino)methyl]sulfanyl-2-(*R*)-[9-(*S*)-hydroxydodecanoyl]aminopropanoate (34).** Using the conditions as described for **17**, THP-protected alcohol **10** (200 mg, 0.671 mmol) and Cys(Acm)-TMSE (235 mg, 0.805 mmol) were coupled. After flash chromatography (50% EtOAc in heptane) the intermediate product (305 mg, 0.625 mmol, 79%) was obtained. $^1$H−NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1H, NHAc), 6.56 (d, *J* = 7.2 Hz, 1H, NH), 5.88−5.74 (m, 1H, C=CH), 5.10−5.04 (m, 2H, $C=CH_2$), 4.77−4.65 (m, 2H, CαH / CH-THP), 4.48−4.29 (m, 2H, $SCH_2N$), 4.29−4.21 (m, 2H, $OCH_2$), 3.92−3.49 (m, 9H, THP, C*H*O-THP), 3.11−2.80 (m, 2H, $CH_2S$), 2.28 (t, *J* = 7.8 Hz, 2H, $CH_2CON$), 2.05 (s, 3H, Ac), 1.73−1.29 (m, 14H, 7 × $CH_2$), 1.04−1.00 (m, 2H,

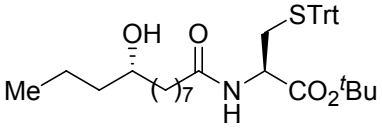


$CH_2TMS$), 0.06 (9H, $SiMe_3$); $^{13}C$–NMR (75 MHz, $CDCl_3$) δ 173.3, 170.5, 170.1, 134.8, 116.6, 97.6, 96.6, 75.1, 64.2. 62.3, 52.7, 42.1, 39.5, 34.9, 25.4, 19.7, −1.63. The intermediate product was dissolved in MeOH (5 mL) and treated with pTSA (10 mg) for 18h at ambient temperature. After further purification using flash chromatography (75% EtOAc in heptane) product **34** (50 mg, 0.10 mmol, 30%, 2 steps) was obtained. $[\alpha]_D$ = –2.12 (*c* = 1.09, $CH_2Cl_2$); $^1H$–NMR (300 MHz, $CDCl_3$) δ 7.17 (d, *J* = 7.4 Hz, 1H, NHAc), 6.77 (d, *J* = 7.6 Hz, 1H, NH), 5.85–5.76 (m, 1H, C*H*=), 5.13–5.07 (m, 2H, $=CH_2$), 4.4-4.69 (m, 1H, CαH), 4.44–4.28 (m, 2H, $SCH_2N$), 2.28 (t, *J* = 7.4 Hz, 2H, $CH_2CON$), 2.02 (s, 3H, NHAc), 1.65–1.29 (m, 14H, $CH_2$), 1.02–0.99 (m, 2H, $CH_2Si$), 0.04 (s, 9H, $SiMe_3$); $^{13}C$–NMR (75 MHz, $CDCl_3$) δ 173.3, 170.5, 134.3, 117.4, 70.4, 64.2, 52.7, 41.7, 36.5, 36.2, 33.9, 28.9, 25.3, 22.9, 17.3, 1.6; IR ν 3265, 3066, 2928, 2854, 1727, 1658, 1536, 1247, 1169, 840 $cm^{-1}$; MS (*m/z*): $C_{23}H_{44}N_2O_5SSi$ $(M + H)^+$ 489, 461, 390, 372, 300, 194, 95, 73.

**2-(1,1,1-Trimethylsilyl)ethyl 3-[(acetylamino)methyl]sulfanyl-2-(*R*)-[9-(*R*)-(3-[(acetylamino)methyl]sulfanyl-2-(*R*)-[(*tert*-butoxycarbonyl)amino] propanoyloxy)dodecanoyl]aminopropanoate (35).** Using the conditions as described for **25**, 3-acetylamino-methylsulfanyl)-2-(*R*)-*tert*-butoxycarbonylaminopropionic acid and **34** were coupled. After flash chromatography (75% EtOAc in hexane) desired product **35** (17 mg, 22 μmol, 29%) was obtained. $^1H$–NMR (300 MHz, $CDCl_3$) δ 6.93–6.78 (d, *J* = 8.0 Hz, 2H, 2×NHAc), 6.69 (d, *J* = 8.0 Hz, 1H, NH), 5.81–5.70 (m, 1H, CH=C), 5.47 (d, *J* = 7.6 Hz, 1H, NHBoc), 5.12–5.07 (m, 2H, $CH_2$=), 4.95 (m, 1H, CαH), 4.77–4.73 (m, 1H, CαH), 4.46–4.36 (m, 4H, 2 × $SCH_2N$), 4.26–4.21 (m, 2H, $CH_2O$), 2.04 (s, 3H, Me), 2.03 (s, 3H, Me), 1.66–1.31 (m, 16H, $CH_2$), 1.47 (s, 9H, Boc), 1.06–1.01 (m, 2H, $CH_2Si$), 0.06 (s, 9H, $SiMe_3$); MS (*m/z*): $C_{34}H_{62}N_4O_9S_2Si$ $(M + H)^+$ 763, 663, 593, 307, 289, 279, 154, 136, 120, 107, 89, 73, 57.




***tert*-Butyl 2-(*R*)-[(9-(*R*)-hydroxydodecanoyl)amino]-3-(tritylsulfanyl) propanoate (36)**. DCC (2.84 g, 13.0 mmol), $^t$BuOH (1.30 g, 17.6 mmol) and CuCl were stirred for 3 days. After dilution using $CH_2Cl_2$ (30 mL), $H_2N$-Cys(Trt)-OH (1.00 g, 2.8 mmol) was added and the resulting suspension was stirred for 1 h. Concentration to ~5 mL and filtration yielded the crude *tert*-butyl ester of cysteine which was directly used in the next step. To a stirred solution of acid **10** (66 mg, 0.31 mmol) in $CH_2Cl_2$ (5 mL) were added DiPEA (125 μL, 0.73 mmol),



crude $H_2N$-Cys(Trt)-O$^t$Bu (200 mg, 0.48 mmol) and BOP (134 mg, 0.31 mmol) and the resulting mixture was stirred overnight. After evaporation and further purification using flash chromatography (3% MeOH in $CH_2Cl_2$) **36** was obtained as an amorphous solid (110 mg, 0.18 mmol, 58%); $^1$H−NMR (400 MHz, $CDCl_3$) δ 7.46−7.43 (m, 6H, Ar), 7.31−7.25 (m, 6H, Ar), 7.24−7.18 (m, 3H, Ar), 5.98 (bd, *J* = 7.3 Hz, 1H, NH), 5.58−4.52 (m, 1H, CαH), 3.64−3.55 (m, 1H, C*H*OH), 2.56 (ddd, *J* = 5.6 Hz, 12.6 Hz, 16.9Hz, 2H, $CH_2S$), 2.16 (t, *J* = 7.4 Hz, 2H, $CH_2CON$), 1.71−1.37 (m, 16H, 8 × $CH_2$), 1.43 (s, 9H, $CMe_3$), 0.92 (t, *J* = 6.7 Hz, 3H, Me); $^{13}$C−NMR (75 MHz, $CDCl_3$) δ 172.0, 169.2, 144.0, 129.2, 127.6, 126.5, 82.4, 71.5, 51.3, 39.7, 37.5, 36.5, 34.4, 34.0, 29.5, 29.3, 28.0, 25.7, 25.0, 19.0, 14.2.

## *6.9 References and notes*


1 Oxitocin is a mammalian nonapeptidehormone that controls mammary and uterine smooth muscle contraction, has neurotransmitting properties in the central nervous system, and displays autocrine and/or paracrine functions in the ovaries and testes.

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8 Upon filtration thermal decomposition of the borohydride can occur.

9 H. Helferich, Schaefer, *Chem. Ber.* **1924**, 1914.

10 A. J. H. Nollet, C. M. Hutting, U. K. Pandit, *Tetrahedron* **1969**, *25*, 5971.

11 Fluorocyanuric acid was used to avoid steric hindrance.

12 The hydrogenation step was scheduled in a later stage of the synthesis.

13 Initial esterfication studies failed when acid chlorides, mixed anhydrides and Mitsunobu-coupling conditions were applied.

14 All spectroscopic data were in accordance with previously reported literature values.

15 The racemic product was prepared as a reference compound via addition of allylMgBr to the aldehyde. Chiral HPLC revealed no trace of the other enantiomer.

16 Grolsch beer bottles ('beugelflessen') proved to be excellent pressure flasks.

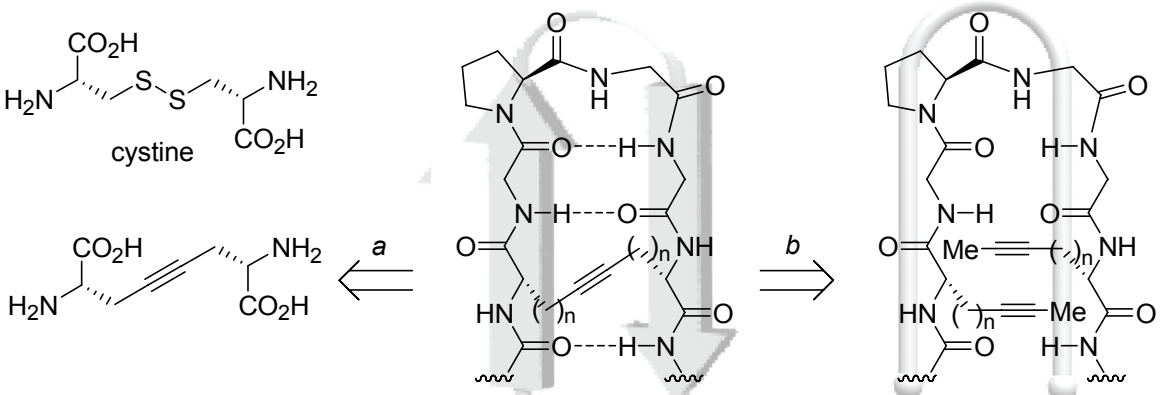
17 Due to the formation of rotamers, the only useful information that could be derived from the $^1$H–NMR spectrum was the absence of the olefinic protons.

# *SUMMARY*


Proteins are Nature's catalysts which continuously drive the metabolism of all living creatures. DNA decoding gives rise to specific linear amino acid chains, which fold themselves or influence by other proteins into more complex ensembles, namely proteins. The folding process leads to frequently encountered patterns among which coiled α-helices and flat ß-sheets stand out as the most important ones.

The α-helices and ß-sheets are relevant because of the relationship between their structure and biological activity. These secondary structures are often found in areas of the proteins where recognition and binding take place.

This thesis aims at developing methods for introducing conformational restriction in ß-turns, the turn elements present in ß-sheets, as exemplified in Scheme 1. A conformationally restricted peptide might either be formed *via* incorporation of a bridging diamino acids in a growing peptide chain (path a), or *via* covalent bond forming processes after folding into a turn structure (path b).

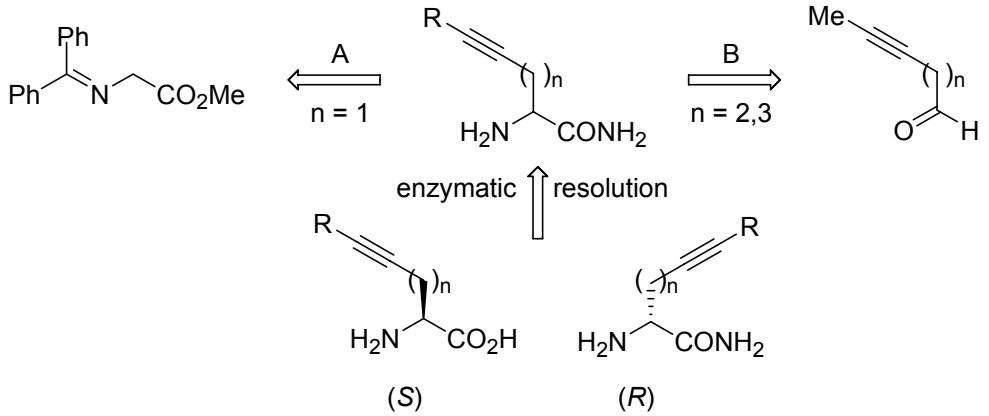



**Scheme 1**. *Two conceptually different pathways to introduce conformational restriction in ß-turns.*

In **Chapter 1** an overview is given of the application of ring-closing olefin metathesis in order to prepare constrained peptides. Advantages and drawbacks of ring-closing metathesis reactions in oligopeptides are highlighted. The chapter ends with several examples of stabilized peptides which possess retained or even increased biological activity.

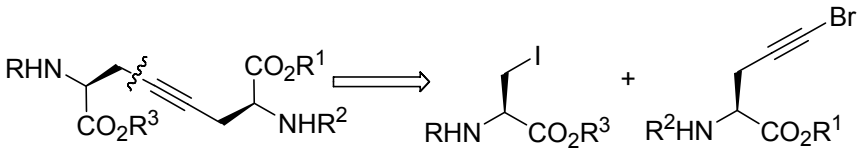
**Chapter 2** describes the chemoenzymatic synthesis of enantiopure acetylene-containing amino acids. Practical synthetic pathways are detailed to pepare amino acid amides, which form an extension of research that has been previously carried out in our group. Enzymatic resolution on these substrates, involving an aminopeptidase that selectively hydrolyzes the naturally configured (*S*)-amino

acid amides, then leads to the enantiomerically pure amino acids which are used throughout this thesis.



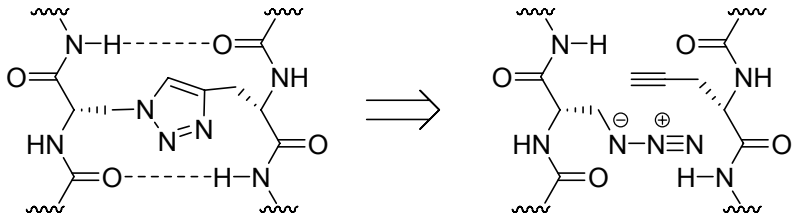

**Scheme 2**. *Chemoenzymatic approach to prepare enantiopure amino acids.*

In **Chapter 3**, methodology involving the coupling of organozinc/copper compounds with halogenated acetylenes is investigated. This has led to the synthesis of a series of new acetylene-containing amino acid derivatives. Furthermore, the strategy has been applied to the synthesis of conformationally restricted diamino acids that may serve as isosteres of cystine in peptide structures.



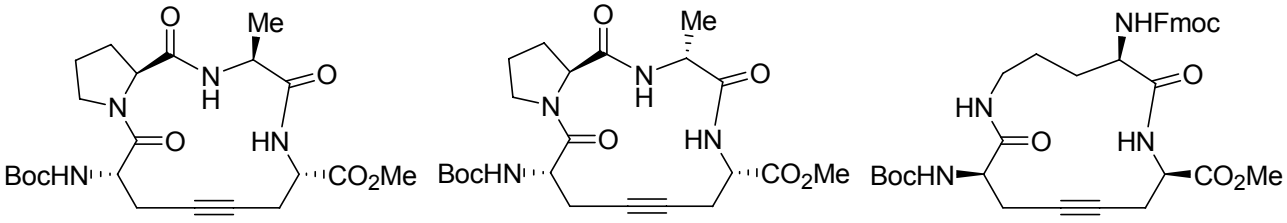

**Scheme 3**. *Construction of conformationally rigid isosteres of cystine via organozinc/copper–bromoacetylene couplings.*

**Chapter 4** involves the application of inter- and intramolecular copper-catalyzed 1,3-dipolar cycloaddition reactions (click-reactions) to introduce conformational restriction in peptide strands. Reaction of acetylenic amino acids with a variety of azides resulted in several potentially biologically relevant compounds such as mimics of the amino acid citrulline. However, in our hands, formation of triazoles via intramolecular cycloaddition in peptides remained unsuccessful.



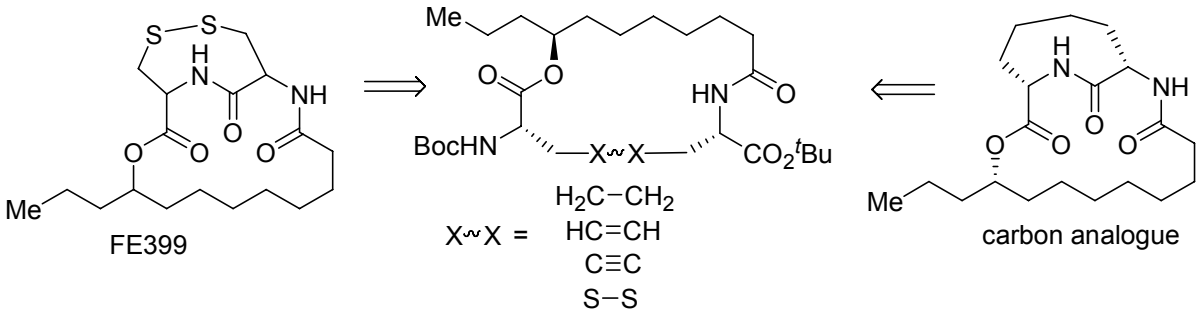

**Scheme 4**. *Concept of intramolecular 1,3-dipolar cycloaddition in peptides.*

In **Chapter 5,** ring-closing alkyne metathesis (RCAM) on suitable acetylene-equipped peptides is the subject of research. The preparation of the required catalyst and several precursor oligopeptides are discussed. Depending on the particular amino acids and the length of the peptide, various constrained oligopeptides were successfully prepared under RCAM conditions. Furthermore, the conformational properties of these constrained peptides were analyzed by NMR and compared to those of comparable cystine-stabilized peptides.




**Scheme 5**. *Several examples of restricted β-turn mimics.*

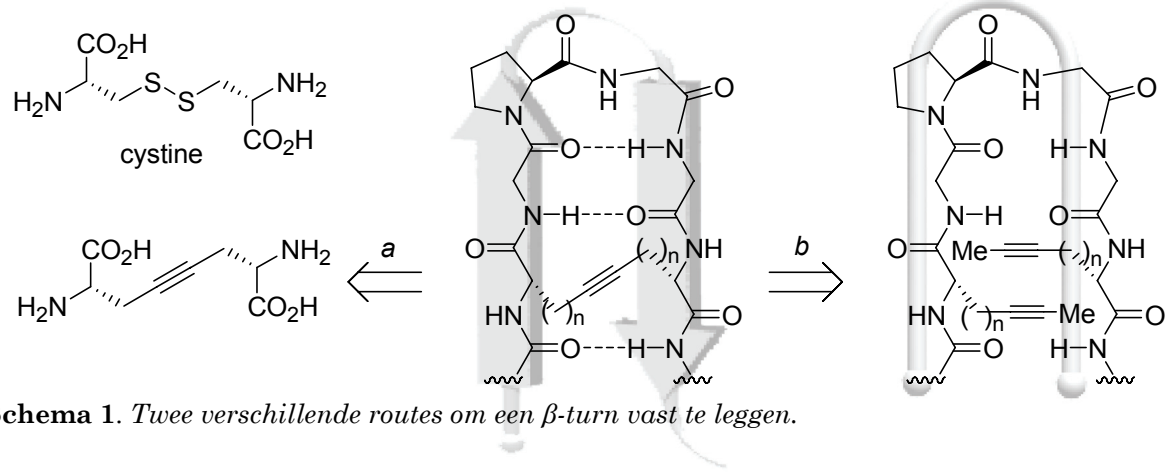
In **Chapter 6**, studies towards a synthesic route of the natural product FE399, containing a cystine bridge, are described, as well as towards carbon analogues. While ring-closing alkyne metathesis failed to give one of the desired carbon mimics, ring-closing olefin metathesis eventually led to a carbon isostere of the natural product. Unfortunately, we were unable to prepare the natural product itself within the timeframe of this thesis.




**Scheme 6**. *Retrosynthetic approach to FE399 and a carbon analogue.*

# *SAMENVATTING*

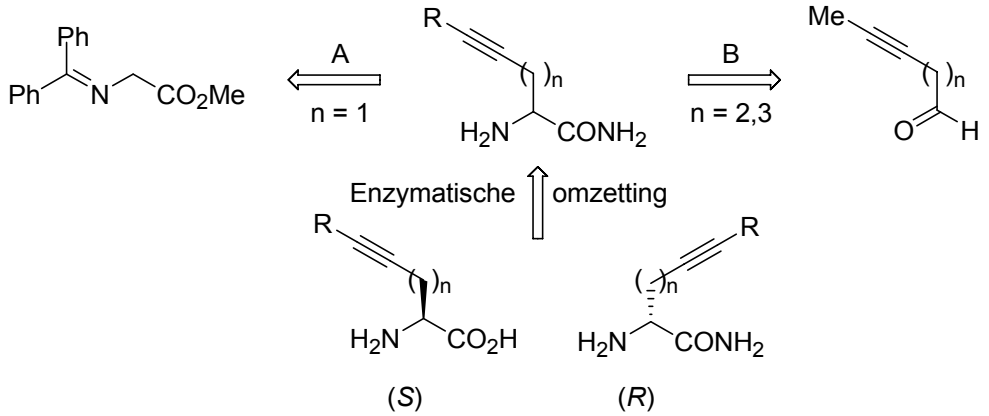
Eiwitten zijn de natuurlijke katalysatoren die het metabolisme van alle levende wezens aansturen. DNA-codering genereert specifieke aminozuurketens, die zichzelf of onder invloed van andere eiwitten in complexe eiwitstructuren vouwen. Dit vouwproces leidt tot frequent terugkerende secundaire structuurelementen waarvan de α-helix en het ß-sheet de meest belangrijke zijn.
De α-helix en het ß-sheet zijn belangrijk vanwege de relatie tussen hun structuur en de daarmee samenhangende biologische activiteit. Dit soort secundaire structuren wordt voornamelijk aangetroffen in eiwitregionen waar herkenning en binding plaatsvinden.
In dit proefschrift wordt aandacht besteed aan de ontwikkeling van nieuwe methoden voor het conformationeel vastleggen van de ß-turn binnen de ß-sheets, zoals getoond in Schema 1. Een conformationeel vastgelegde eiwitketen kan worden verkregen via het inbouwen van een bruggend diaminozuur in een groeiende peptideketen (route a) of via covalente bindingsvorming met behulp van een geschikte katalytische reactie na het invoeren van de afzonderlijke aminozuren in de peptideketen (route b).




**Schema 1**. *Twee verschillende routes om een β-turn vast te leggen.*

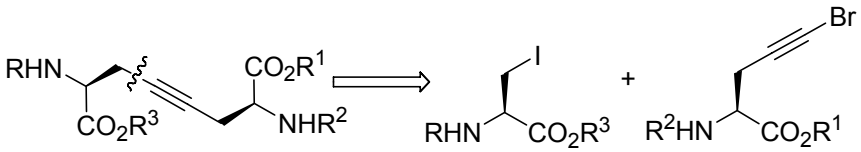
In **Hoofdstuk 1** wordt een overzicht gegeven van de actuele ontwikkelingen om via ringsluitingsalkeenmetathese (RCM) conformationeel vastgelegde eiwitten te synthetiseren. De voor- en nadelen van deze metathesereacties in korte aminozuurketens worden weergegeven. Het hoofdstuk eindigt met enkele voorbeelden van conformationeel vastgelegde peptideketens waarvan de biologische activiteit gelijk is gebleven of zelfs verbeterd.
**Hoofdstuk 2** beschrijft de chemoenzymatische synthese van enantiomeerzuivere acetyleenhoudende aminozuren. Praktische syntheseroutes zijn beschreven, die een uitbreiding vormen van eerder binnen de Rutjes groep op dit gebied

ontwikkelde kennis. Enzymatische hydrolyse van racemische aminozuuramiden met behulp van een aminopeptidase, geeft selectief de natuurlijke geconfigureerde aminozuren, terwijl de niet-natuurlijk geconfigureerde aminozuuramiden intact blijven. Deze aldus verkregen aminozuren worden vervolgens gebruikt in de rest van dit proefschrift.



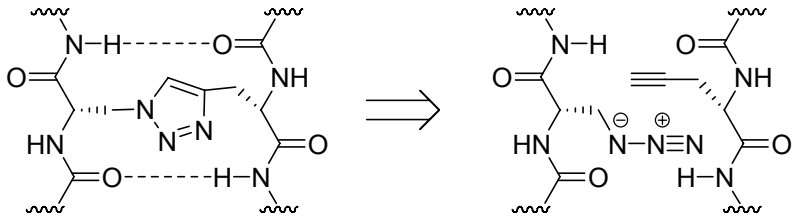

**Schema 2**. *Chemoenzymatische benadering voor de synthese van enantiomeerzuivere aminozuren.*

In **Hoofdstuk 3** wordt een methode onderzocht om geactiveerde organo-zink/koper verbindingen te koppelen met verschillende gehalogeneerde acetylenen. Deze methode leidt tot de synthese van enkele nieuwe acetyleenhoudende aminozuren. Ook wordt deze methode onderzocht voor de synthese van starre diaminozozuren die mogelijk als brug kunnen worden ingebouwd in korte aminozuurketens.



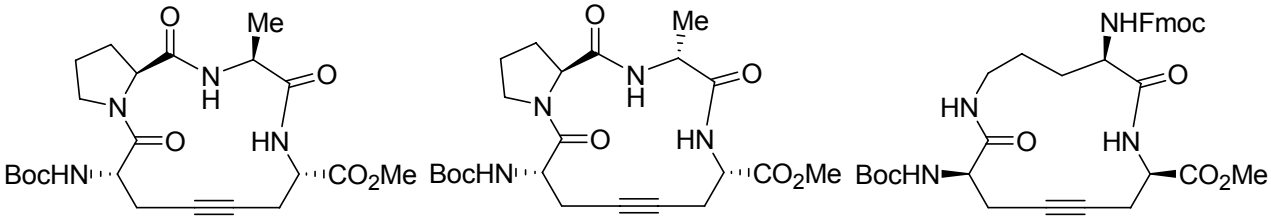

**Schema 3**. *De opbouw van starre diaminozuren via geactiveerde organo-zink/koper verbindingen.*

**Hoofdstuk 4** behandelt de toepasbaarheid van inter,- en intramoleculaire kopergekatalyseerde 1,3-dipolaire cycloaddities (zogenaamde 'klik-reacties') tussen acetylenen en organische aziden om aminozuurketens conformationeel vast te leggen. De reactie tussen een acetyleenhoudend aminozuur met verschillende aziden levert enkele mogelijk biologisch relevante verbindingen op, zoals mimics van het aminozuur citrulline. Ondanks deze intermoleculaire voorbeelden is de vorming van de triazool via intramoleculaire cycloaddities voor ons weinig succesvol gebleken.



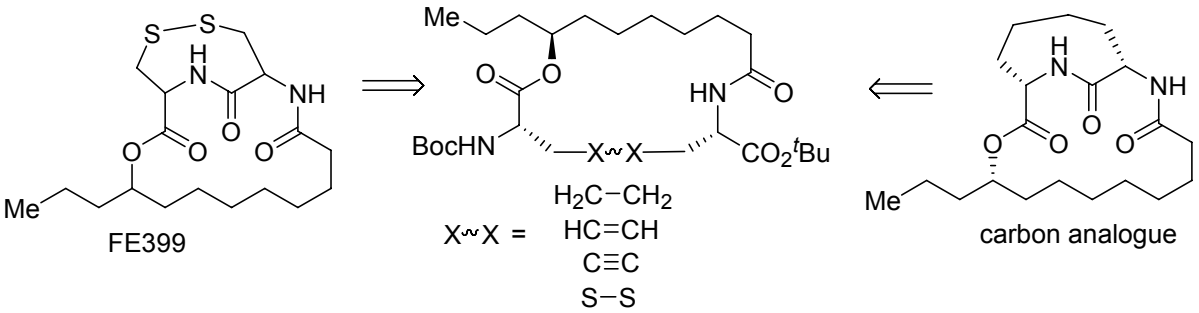
**Schema 4**. *Het concept van intramoleculaire 1,3-dipolaire cycloadditie in peptiden.*

In **Hoofdstuk 5** is ringsluitingsalkynmetathese (RCAM) op acetyleen-houdende oligopeptiden het onderwerp van onderzoek. De synthese van de benodigde wolfraamkatalysator en een reeks precursormoleculen wordt besproken. Afhankelijk van de ingebouwde acetyleenhoudende aminozuren en de lengte van de aminozuurketen zijn verschillende vastgelegde eiwitstructuren gemaakt via RCAM. Deze conformationeel vastgelegde structuren zijn vervolgens met behulp van NMR-technieken vergeleken met vergelijkbare natuurlijke structuren.



**Schema 5**. *Enkele voorbeelden van conformationeel vastgelegde β-turn mimics.*

**Hoofdstuk 6** behandelt onderzoek naar een syntheseroute van de natuurstof FE399 en koolstofbevattende analoga daarvan. Waar RCAM niet succesvol bleek bij het verkrijgen van de gewenste analoga, leidde RCM tot een gewenst koolstofanalogon. De synthese van de natuurstof zelf kon niet worden volbracht binnen het tijdsbestek van dit promotieonderzoek.



**Schema 6**. *Retrosynthetische benadering van FE399 en een koolstofanalogon.*

# *DANKWOORD / ACKNOWLEDGEMENTS*

En nu, na al die jaren van chemie achter de zuurkast
Is het werk volbracht en houdt u de samenvatting in uw hand vast
Nu hoef ik slechts een uitgebreid en kloppend dankwoord te schrijven
Dat bij voorkeur ook in uw gedachten zal blijven
Een die qua werk en moeilijkheden bij de afgelopen jaren past

Ruim aandacht uiteraard voor mijn promotor: Floris
'U' (want zo noemde ik je steeds dus)
Telefoneerde als enige naar mij in het lab van prof. Hanessian
Je vroeg of ik interesse had om naar Nijmegen te gaan
En dat was het begin van een goede en leuke samenwerking
Synthese doen in een stimulerend klimaat, zonder enige beperking

En zoals het op de Katholieke Universiteit betracht
Was er een creatieve en ondersteunende kracht
De dames Jacky/Maria en Desiree, beoefenaars van de werkelijke macht
Richard Blaauw, wiens dagelijkse advies ik nog steeds vertrouw
Floris van Delft als mijn eigenwijze wederhelft
Maar ook Prof. Hans Schoemaker van DSM had in dit manuscript een stem
En zonder Mark Overhand was dit proefschrift zeker gestrand.

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Het onafscheidelijke duo Cyrus & Mona, dat zelfs samen promoveerde
De kleine Christien die met veel plezier de HPLC analyseerde
En ook Koen, toen nog student van Hester (het intelligente blontje)
Allemaal dronken we na zware 'probleemavonden' gezellig een rondje

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Weird Lee as (pseudo / semi)partner in crime for our project of STW
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Tenslotte Jeffrey en Dennis voor het helpen met meer dan alleen de Kaisertest

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Zoals Claudia op precies dezelfde 'golflengte' afgesteld stond
En Guuske (ohoh) op het congres in Riga met een grote Oranje toeter
Staat nu samen met Korinde en Suzanne als Floris' Angels op de computer
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De gamefanaten annex polymeerboeren Henri en Joost
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Een tweetal Daniels met allebei met een heel dik Spaans accent
Het huiselijke welkome gevoel bij Chiralix door Denis, Peter en Jasper
De iWings-ontwikkelingsploeg Ton en (met een A) Kaspar
De mensen van eigenlijk voor mijn tijd: Sander, Rene en lovely Corrine
En natuurlijk Bertus, die pas zeer laat zijn proefschrift het licht liet zien

Met veel plezier denk ik aan professor Nolte en vooral zijn kroost
Altijd leuke grappen van Aurelie, Dennis, Marta, Gerald,, Irene, Johan, Joost,
Femke, Matthijs, Mark, Ruud en Vera maar dat als team zeer goede resultaten oogst

Ook de veelzijdige studenten van toen (nu aio) Jasper en Pieter verdienen hier een lijn
Evenals de buurmannen Rene, Leon en studenten, specialisten van het hoge drukterrein
Voorts de stille doch aanwezige krachten van Joris en Martijn
En de 'vriendelijke' Roel en Sander zullen inmiddels ook wel eiwitgek zijn
De inbreng van onze postdocs, Pjotr en Sjors was zeker niet klein
Maar studenten kunnen ook goed werken, zoals Toine en Bram op de katalysefrontlijn.
Of anderzijds was het met Jorge (de Tour) en Suzanne (met gitaar) wel fijn.
Ou finalement mes amis Eric et Jean-Christophe pour le temps chez le pay du paturain

Sowieso is chemie niet echt mogelijk zonder ondersteunend personeel
Technici zoals Ad en z'n NMR hebben in dit proefschrift zeker hun aandeel
En anders wel Helene of de andere Peter van de 'massa'spectrometer
Uren zijn ook aan het bestellen van chemicaliën besteedt door Wim, Chris en Peter
Niets kreeg het netwerk plat dankzij Gerry en Pieter; bedankt voor de zorgeloze e-mail

Groot respect voor de plek waar ik mijn zuurkast vond
Een eeuwenoud gebouw, groezelig, donker doch toch verlicht
Betonrot, vreemde rioollucht en ook de kakkerlakken maakte het vrij bont
Ondanks dat werd er toch in een goede sfeer wetenschap onderricht
Uiteindelijk, na ongeveer 30 jaar gesteggel, ging het toch tegen de grond
Wij trokken in een nieuw gebouw, waar echter cubicles waren opgericht...

Mijn naam is Maarten IJsselstijn
En zou zonder mijn ouders en lieve zus niet tot hier gekomen zijn
Maar nu begin ik met een nieuwe baan in het mooie Grun'n
En kan dit proefschrift eindelijk de boekenkast in.

# *CURRICULUM VITAE*

De schrijver van dit proefschrift werd geboren op 23 september 1976 te Bovenkarspel. In juni 1993 behaalde hij zijn HAVO diploma aan de Openbare Scholengemeenschap West-Friesland, gevolgd in juni 1995 door het VWO diploma. In september van dat jaar begon hij met de studie scheikunde aan de Universiteit van Amsterdam. Als hoofdvakstage werd in de groep van prof. H. Hiemstra gewerkt aan diverse syntheseroutes naar solanoeclepine A, een potente wekstof van aardappelaaltjes. Voorts werd tussen januari en juni 2000 een bijvakstage volbracht aan de Université de Montréal (Canada) in de groep van prof. S. Hanessian. Het doctoraal examen met als specialisatie Organische Chemie werd in september 2000 gehaald.

Van september 2000 tot en met augustus 2004 was hij werkzaam als Assistent-in-Opleiding/Junior Onderzoeker bij de leerstoelgroep Synthetisch Organische Chemie aan de Radboud Universiteit Nijmegen. Gedurende deze periode heeft hij onder leiding van prof. F. P. J. T. Rutjes het, in dit proefschrift beschreven onderzoek verricht, dat mede door STW werd gefinancierd,

Van november 2004 tot oktober 2005 is postdoctoraal onderzoek verricht onder leiding van Dr. B. Rousseau en Dr. J.-C. Cintrat aan het Commissariat a l'Energie Atomique' te Parijs (Frankrijk).

Vanaf 1 juli 2006 is Maarten IJsselstijn werkzaam als research scientist bij Syncom in Groningen.